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Interactions between EPS-producing *Streptococcus thermophilus* strains in mixed yoghurt cultures

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Mixed cultures of different EPS-producing *Streptococcus thermophilus* strains in combination with a *Lactobacillus delbrueckii* subsp. *bulgaricus* strain with negligible EPS-production were used for yoghurt production. The yoghurt texture was characterised with respect to sensory, rheological and microstructural properties and the EPS-concentrations were determined. The cultures resulted in yoghurts with highly different texture properties, and positive interactions between certain *Streptococcus thermophilus* strains were observed. The underlying properties of yoghurt texture are multidimensional, but a number of microstructural characteristics were apparent in the yoghurts with the highest mouth thickness, creaminess and viscosity. A strong protein network, not too dense and with medium size pores containing EPS, seems associated with these properties. The presence of capsular polysaccharides (CPS) also appeared to be beneficial as did a combination of EPS types, which were distributed differently in the protein network (in serum pores, respectively in association with protein). Obviously, a certain concentration of EPS must be present to provide for these effects on yoghurt texture, but other factors than concentration per se seem more important.

Keywords: exopolysaccharides, yoghurt texture, sensory profile, ropiness, confocal laser scanning microscopy, microstructure.

Exopolysaccharides (EPS) are produced by many lactic acid bacteria cultures during fermentation (Cerning, 1990) and numerous studies have documented that EPS have highly significant effects on the texture properties of fermented milk products (Hassan et al. 1996; Bouzar et al. 1997; Hess et al. 1997; Rawson & Marshall, 1997; Laws & Marshall, 2001; Ruas-Madiedo et al. 2002; Ruas-Madiedo & Zoon, 2003). EPS are high molecular weight carbohydrates with a backbone of repeated subunits of monosaccharides. The EPS-concentration found in fermented milk products is normally between 50 and 600 mg l^{-1} depending on the culture and the fermentation conditions (De Vuyst et al. 2001) as well as the method used to isolate the EPS (Ruas-Madiedo & de los Reyes-Gavilán, 2005).

The presence of EPS often imparts a ropy character to the fermented product (Cerning, 1990), and EPS-producing bacterial strains are often termed 'ropy strains'. Ropiness, however, does not always correlate with the concentration of EPS, which makes 'ropy strains' an imprecise term for EPS-producing strains. The term 'mucoid strains' has also been used in literature referring to strains that produce colonies with a slimy and glistening appearance, but are not able to produce strands, i.e. are non-ropy (Ruas-Madiedo & de los Reyes-Gavilán, 2005). In addition to ropiness, milk products fermented with EPS-producing cultures obtain high viscosity and high creaminess as well as increased water binding capacity (Rawson & Marshall, 1997). Hassan et al. (1996) found that products where the EPS were situated in capsules closely associated with the bacteria (capsular polysaccharides, CPS) were less ropy than products with the EPS distributed in the total product volume.

Several studies have shown that there is no clear relation between the concentration of EPS and the viscosity of fermented milk products (Van Marle & Zoon, 1995; Dupont et al. 2000; Petry et al. 2003). Marshall & Rawson (1999) concluded that the amount of EPS was not important for the rheological properties of yoghurts, and

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suggested that the type of EPS and their interaction with milk proteins is the determining factor. Ruas-Madiedo et al. (2002) found a positive correlation between intrinsic viscosity of EPS from different *Lactocccus lactis* subsp. *cremoris* strains in aqueous solutions and Posthumus viscosity of milk fermented with these strains. The molar mass of EPS as well as the radius of gyration of the EPS molecule has also been shown to influence the viscosity of fermented milk products (Faber et al. 1998; Ruas-Madiedo et al. 2002). However, a comparison of the available literature on EPS studies suggests that large strain-to-strain variation exists within the group of EPS-producing strains regarding their effects on sensory and rheological properties in fermented milk products. Thus no consistent correlations between EPS concentration, sugar composition, molecular structure and the resulting sensory and rheological properties seem to have been found so far (Jolly et al. 2002).

EPS-containing fermented milk products have also been subjected to microstructural studies. Confocal laser scanning micrographs (CLSM) of yoghurt fermented with an EPS-producing culture before and after shear, respectively, were presented by Hassan et al. (2002). Stirring induced the segregation of protein and EPS, causing the EPS to self-interact and collect in large agglomerates, which was assumed to account for the increased ropiness found in the stirred product.

Bouzar et al. (1997) found that the viscosity and smoothness of yoghurts could be improved by starter cultures containing a mix of different EPS-producing strains. Hassan et al. (1996) found that a mixed culture of *Lb. bulgaricus* and *Strep. thermophilus* mucoid, non-ropy strains exhibited greater apparent viscosity than non-mucoid, non-ropy strains. Rawson & Marshall (1997) reported that the combination of a ropy strain of *Lb. delbrueckii* subsp. *bulgaricus* and a non-ropy strain of *Strep. thermophilus* produced a viscous product, which recovered its viscosity after destructive testing, whereas the combination of two ropy strains resulted in a product, which lost its viscosity more rapidly and could not recover the lost viscosity so well. Thus it seems that not only the EPS production properties of single strains but also interactions between strains determine the performance of a mixed culture. This point is important to keep in mind, as cultures applied in the production of commercial dairy products are normally mixed cultures of two or more strains.

In the present study, several mixed cultures containing different EPS-producing *Strep. thermophilus* strains were used for yoghurt production. The EPS-producing strains have previously been found to fall into two different groups with respect to EPS distribution in the yoghurt gel as well as sensory properties of yoghurts. In this study sensory, rheological and microstructural properties as well as EPS-concentration of the mixed culture yoghurts were analysed in order to unravel interactions between the strains.

Materials and methods

Bacterial cultures

Five different EPS-producing *Strep. thermophilus* strains, CHCC-5581 (ST1), CHCC-2389 (ST2), CHCC-3021 (ST3), CHCC-4239 (ST4) and CHCC-5086 (ST5), and one *Lb. delbrueckii* subsp. *bulgaricus* strain with negligible EPS-production (CHCC-769) were chosen from the Chr. Hansen Culture Collection (CHCC). Throughout this paper the short strain names ST1, ST2, ST3, ST4 and ST5 will be used in the text and figures. All strains were DVS (direct vat set) material. Yoghurts were produced using either one ST strain in combination with the *Lb. bulgaricus* strain or a mixture of two ST-strains in combination with the *Lb. bulgaricus*. A total of 15 different cultures were applied to yoghurt production (5 single ST cultures plus all ten possible two-ST mixtures). All cultures consisted of 20% *Lb. bulgaricus* and 80% ST (in the mixed-ST cultures this was 40% of each ST).

Yoghurt production

Yoghurts were produced from reconstituted (120 g/l) medium heat skimmed milk powder (www.procudan.dk), which had been heat-treated at 90 °C for 20 min. The skimmed milk was cooled to 43 °C, inoculated with a total of 0·2 g/l DVS culture. Approximately half of the inoculated milk was poured into 125 ml beakers and incubated at 43 °C in a thermo chamber (set yoghurts); while the rest of the inoculated milk was fermented in a 4 l steel bucket in a 43 °C water bath (for stirred yoghurt production). At pH 4·70±0·02 the set yoghurts were removed from the thermo chamber and stored at 5 °C. At pH 4·60±0·03 the yoghurt curd fermented in water bath was stirred 30 times with a hand stirrer and subsequently stirred 30 seconds at medium speed (step 5 out of 9) using a Silverson L4R mixer (www.silverson.com). Hereafter the stirred yoghurt was cooled in ice water until the temperature in the middle of the sample reached 25 °C. The yoghurt was poured into 3 l plastic containers and stored at 5 °C. Sensory, rheological and microstructural analyses were carried out after 5 d storage. The 15 different yoghurts were produced in two replicates on 6 different production days.

Sensory evaluation

Eight experienced assessors evaluated the sensory properties of the yoghurts. During three training sessions the assessors developed a vocabulary of 11 texture attributes; of these 6 attributes described the set yoghurts and 5 attributes described the stirred yoghurts (Table 1). Mouth thickness and creaminess were evaluated in both set and stirred yoghurt types. Ropiness of the separated serum in set yoghurts as well as ropiness of the stirred product was evaluated. Gel firmness was also evaluated in both yoghurt types but in the set yoghurts the evaluation included a

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Table 1. Attributes and definitions used for sensory profiling of yoghurts

Set yoghurt	
Serum	The volume of separated serum on the yoghurt surface
Serum ropiness	The spoon is placed on the surface and pulled up and the ropy property is evaluated. The longer the thread becomes before it breaks the ropier the product
Clean cut	A spoonful of yoghurt is removed and the edges of the cut are evaluated: absent = very rough; high = smooth
Gel firmness	A spoonful of yoghurt is placed on the untouched surface of the remaining yoghurt and a slight pressure is applied with the spoon. At high gel firmness the yoghurt breaks in flakes (resembling boiled egg white) whereas yoghurt with low gel firmness is soft
Mouth thickness	Perceived as the degree of thickness when swallowing the yoghurt at normal-high eating rate. High mouth thickness corresponds to a thick product that takes a long time to swallow.
Creaminess	The intensity of creaminess (soft and velvety, not watery)
Stirred yoghurt	
Shiny	The shininess is evaluated: absent = gritty, rough; high = shiny, smooth
Gel firmness 2	A spoonful of yoghurt is placed on the untouched surface of the remaining yoghurt and it is observed for how long the structure is retained. High gel firmness corresponds to a long time of structural integrity
Ropiness of yoghurt	A spoonful of yoghurt is pulled from the sample and the ropy property is evaluated. The longer the thread becomes before it breaks the ropier the product
Mouth thickness	Same definition as mouth thickness in set yoghurt
Creaminess	Same definition as creaminess in set yoghurt

pressure with a spoon, which was necessary in order to see a difference between the products (see definitions in Table 1.) The set yoghurts were served in production beakers; the stirred yoghurts were poured into 100 ml beakers and rested approximately 2 h prior to the evaluation. All yoghurts were tempered to 13 °C, and during the evaluations the cups were placed in thermo-cups to avoid heating of the samples. A 13 cm non-structured scale was used for evaluation, with the ends corresponding to absent and high intensity of each attribute. Samples were coded with three-digit numbers and served in randomised order following Latin square designs.

Microstructure

The microstructure of the yoghurts was observed at room temperature (20 °C) using an inverted CLSM Leica TCS SL (www.leica-microsystems.com) fitted with an Ar and two HeNe lasers and a 20× objective. Samples of the set yoghurts were carefully cut out from the middle of the production beaker and stirred yoghurt samples were taken with a spoon. The samples were stained with fluorescein-isothiocyanate (FITC) from Molecular Probes (www.probes.com) and excited by the 488 nm wavelength laser and emission was collected in the range 500–535 nm. Two images of each yoghurt were recorded at a sample depth of approximately 5–10 µm. Furthermore, the five single strains were tested for production of capsular polysaccharides (CPS) using the procedure described by Hassan et al. (1995). Pure strains were grown in milk over night at 43 °C, diluted in 43 °C warm milk and immediately subjected to CLSM using a 63× objective. The samples were observed in reflectance mode, collecting reflection around the 488 nm laser (456–509 nm) and the presence of non-reflective areas surrounding the bacteria cells observed for some strains indicated that these produced CPS.

Rheology

The rheological properties of the stirred yoghurts were characterised by a flow curve obtained using a Stresstech rheometer from Reologica Instruments (www.reologica.se) fitted with a bob and cup measuring system. Prior to the analysis the yoghurts were brought to a temperature of 13 °C and the measurements were carried out at this temperature. After a 200 s equilibrium time, a flow curve measuring shear stress as a function of shear rates from 0·3 to 300 s^{-1} (up and down sweeps) was recorded in 42 steps with logarithmic intervals. Hysteresis loop area between the upward and downward flow curve was calculated.

Siphon test

Tubeless siphon test, which provides an empirical measurement of extensional viscosity, was performed on the stirred yoghurts in order to evaluate how well the results from the tubeless siphon test reflect the ropiness perceived by sensory panellists. A siphon system was assembled by connecting a water suction pump and a plastic tube (length 375 mm/diameter 6 mm). The lower end of the tube was placed 5 mm below the surface of the yoghurt sample, and vacuum was applied by turning on the water. Yoghurt was aspirated from the sample container until the siphon thread broke, and the weight of the aspirated material was calculated as the difference in yoghurt sample weight before and after the test.

Determination of EPS concentrations

After addition of 38 mg pronase (www.boehringer-ingelheim.com) (to remove protein) and 75 µg Na-azide (to kill bacteria) yoghurt samples (75 g) were incubated overnight at 30 °C. The enzymatic reaction was stopped by boiling and hereafter the samples were centrifuged

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(23 000 ***g***, 15 min, 4 °C) to remove cells and protein. Ice cold 96% ethanol (1·5 ml ml^{-1}) and 1·5 ml 5 M-NaCl were added to the supernatant and the samples were left at 4 °C over night to precipitate the EPS. The samples were centrifuged (23 000 ***g***, 30 min, 4 °C) and the precipitate dissolved in 75 ml MilliQ water. The EPS precipitation step was repeated 3 times; however after the third precipitation the EPS were dissolved in only 7·5 ml MilliQ water and frozen prior to further analysis. EPS concentrations were determined by the phenol–sulphuric acid method using glucose as a standard (Dubois et al. 1956). Results were expressed in mg glucose equivalents per litre of yoghurt.

Data analysis

Image analysis of CLSM images. CLSM images (512 × 512 pixels equal to an image size of 750 × 750 µm) were unfolded to vectors containing 512^2 data points ranging from 0 to 256 in value. The Angular Measure Technique (AMT; Andrle 1994) was used to provide an overview of the total information in the CLSM images. An improved AMT-algorithm written for Matlab 6.5 by Johansen et al. (2005) was used. The AMT technique characterises complexity at a given scale, S. In our case, S varied from 1 to 256 pixels and within each cell of a predefined grid of 100 × 100 cells one random point was selected, giving a total of 100^2 A-points. The digitized points that coincide to distance S from point A were then selected as the endpoints B and C. A mean angle, MA(S) is calculated by averaging over the supplementary angle-to-angle BAC of all A-points for fixed S. Low MA values signify low complexity (i.e. areas with similar light intensity), whereas high MA values imply significant complexity (i.e. shifts in light intensity). Mean angles obtained at low S values describe the degree of local variation whereas mean angles at high S values describe the overall (global) variation within an image. Fourteen mean angles representing the degree of variation at respectively small (MA2-MA6), medium (MA100-MA102) and high (MA200-MA202 and MA255-MA257) S-values were selected and included in the ANOVA described in the following section.

ANOVA. Prior to data analysis the sensory data were subjected to a level correction procedure as described by Thybo & Martens (2000) in order to adjust the different assessors' use of the scale. Analysis of variance (ANOVA) of the instrumental and the level corrected sensory data was performed using the SAS 8.02 software (www.sas.com). A general linear model was used to investigate main effects of the 15 different yoghurt cultures. A Least Significance Difference (LSD) test (P=0·05) was carried out for each sensory attribute found to differ significantly between the yoghurts. ANOVA on the AMT parameters was performed separately to allow inclusion of the duplicate images of each yoghurt. The results from the ANOVA were used to support the interpretation based on the multivariate analyses.

Principal Component Analysis and Partial Least Squares Regression. Principal Component Analysis (PCA) and the level correction of sensory data as well as Partial Least Squares Regression (PLS) were performed using the Unscrambler 9.0 software (www.camo.com). The level corrected sensory data, the rheological data and the EPS-concentration of the stirred yoghurts were analysed in one PCA, whereas the level corrected sensory data for set yoghurts were analysed in another PCA. A PLS regression on all mean angles from AMT (stirred yoghurts) versus level corrected sensory data for the same yoghurts was also performed. All analyses were performed with standardized variables.

Results

ANOVA

Table 2 shows the data for those of the level corrected sensory data and instrumental variables that were found to differ significantly among the yoghurts. The standard deviation for each variable (pooled over all samples) and the results of the LSD test also appear in Table 2. Clean cut, gel firmness, creaminess and mouth thickness in set yoghurt did not differ significantly. All 14 selected variables from the AMT described significant differences between the stirred yoghurts (P=0·0011–0·0362) whereas only a few mean angles discriminated significantly among the set yoghurts.

Stirred yoghurts

Figure 1 shows bi-plot (loadings and scores) from PCA on the sensory and instrumental data for the 15 different stirred yoghurts. The first two significant principal components explained a total of 80% of the variation in the data. The loadings of the first and second principal components showed that mouth thickness and creaminess were positively correlated with viscosity and also with loop area. Ropiness was highly positively correlated with the siphon test results (extensional viscosity) indicating that this analysis provided a very good estimation of sensory evaluated ropiness. Principal component one (Pc 1) differentiated yoghurts with high mouth thickness, creaminess, viscosity and loop area (placed to the right in Fig. 1) from those with low mouth thickness and viscosity etc. (placed to the left in Fig. 1). The Pc 2 spanned the variation between yoghurts with high ropiness and shininess and low gel firmness (samples placed at the top of Fig. 1) and those with high gel firmness, low ropiness and shininess (at the bottom of Fig. 1). Ropiness, gel firmness and EPS concentration were, however, described in both components. To some extent the EPS-concentration was positively

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Table 2. A selection of rheological and level corrected sensory data and EPS concentration for yoghurt produced with the 15 different cultures; given in average over two replicates. For each variable the *P*-value for difference between the cultures is given. Cultures marked with the same capital letter were not significantly different according to the Least Significant Difference test ($P=0{\cdot}05$), the letter A is assigned to the sample with the highest level of the given property, B to the next highest level and so forth

					Level corrected sensory data						
					Set yoghurt		Stirred yoghurt				
	Viscosity (pa s) at shear rate 300 s^{-1}	Loop area (Pa s^{-1})	Siphon test (g)	EPS conc. (mg/L)	Serum	Serum ropiness	Shiny	Gel firmness	Ropiness	Mouth thickness	Creaminess
P-value for difference	0·0001	0·002	0·019	0·0009	0·004	0·0001	0·001	0·0001	0·019	0·012	0·09
ST1	$0{\cdot}199^{BCD}$	$6{\cdot}41{\cdot}10^{3AB}$	$30{\cdot}46^{CDE}$	$170{\cdot}0^{A}$	$-2{\cdot}31^{G}$	$-2{\cdot}64^{GH}$	$-1{\cdot}64^{E}$	$2{\cdot}01^{A}$	$-0{\cdot}44^{CDE}$	$-0{\cdot}34^{DEF}$	$0{\cdot}41$
ST2	$0{\cdot}165^{EF}$	$5{\cdot}32{\cdot}10^{3CDE}$	$28{\cdot}64^{CDE}$	$94{\cdot}5^{BCDE}$	$-0{\cdot}70^{DEF}$	$-2{\cdot}85^{H}$	$-1{\cdot}73^{E}$	$0{\cdot}26^{CDEF}$	$-0{\cdot}99^{DE}$	$-0{\cdot}42^{DEF}$	$-1{\cdot}27$
ST3	$0{\cdot}135^{H}$	$4{\cdot}39{\cdot}10^{3F}$	$25{\cdot}08^{E}$	$52{\cdot}2^{EFG}$	$0{\cdot}04^{ABCDE}$	$-0{\cdot}68^{DEF}$	$0{\cdot}44^{AB}$	$0{\cdot}36^{CDE}$	$-2{\cdot}44^{E}$	$-0{\cdot}78^{F}$	$-0{\cdot}99$
ST4	$0{\cdot}181^{DE}$	$5{\cdot}59{\cdot}10^{3BCD}$	$32{\cdot}65^{BCDE}$	$35{\cdot}3^{FG}$	$1{\cdot}14^{A}$	$2{\cdot}27^{ABC}$	$0{\cdot}01^{ABCD}$	$-0{\cdot}59^{EFGH}$	$0{\cdot}31^{ABCD}$	$0{\cdot}05^{CDEF}$	$0{\cdot}21$
ST5	$0{\cdot}208^{AB}$	$5{\cdot}32{\cdot}10^{3CDE}$	$42{\cdot}36^{AB}$	$54{\cdot}9^{EFG}$	$0{\cdot}27^{ABCDE}$	$0{\cdot}73^{BCD}$	$0{\cdot}50^{AB}$	$-0{\cdot}35^{DEFG}$	$1{\cdot}74^{AB}$	$-0{\cdot}48^{DEF}$	$-0{\cdot}41$
ST1+ST2	$0{\cdot}199^{BC}$	$6{\cdot}37{\cdot}10^{3AB}$	$30{\cdot}14^{CDE}$	$120{\cdot}2^{ABCD}$	$-0{\cdot}90^{EF}$	$-2{\cdot}30^{FGH}$	$-0{\cdot}69^{CDE}$	$1{\cdot}64^{AB}$	$-0{\cdot}12^{BCD}$	$0{\cdot}50^{ABCDE}$	$0{\cdot}83$
ST1+ST3	$0{\cdot}190^{CD}$	$6{\cdot}53{\cdot}10^{3A}$	$29{\cdot}06^{CDE}$	$52{\cdot}7^{EFG}$	$-0{\cdot}62^{BCDEF}$	$-1{\cdot}06^{EFG}$	$0{\cdot}06^{ABC}$	$0{\cdot}68^{C}$	$0{\cdot}60^{ABCD}$	$1{\cdot}49^{AB}$	$1{\cdot}25$
ST1+ST4	$0{\cdot}200^{BC}$	$6{\cdot}35{\cdot}10^{3AB}$	$30{\cdot}30^{CDE}$	$140{\cdot}6^{AB}$	$-0{\cdot}66^{CDEF}$	$-1{\cdot}14^{EFG}$	$0{\cdot}10^{ABC}$	$1{\cdot}72^{AB}$	$1{\cdot}19^{ABC}$	$1{\cdot}13^{ABC}$	$0{\cdot}74$
ST1+ST5	$0{\cdot}223^{A}$	$6{\cdot}20{\cdot}10^{3ABC}$	$38{\cdot}33^{ABC}$	$128{\cdot}5^{ABC}$	$-1{\cdot}46^{FG}$	$-0{\cdot}41^{DE}$	$-0{\cdot}40^{BCD}$	$0{\cdot}80^{BC}$	$0{\cdot}77^{ABCD}$	$1{\cdot}75^{A}$	$1{\cdot}25$
ST3+ST2	$0{\cdot}139^{GH}$	$4{\cdot}58{\cdot}10^{3EF}$	$30{\cdot}16^{CDE}$	$51{\cdot}7^{EFG}$	$-0{\cdot}04^{ABCDE}$	$-0{\cdot}53^{DE}$	$0{\cdot}34^{ABC}$	$-0{\cdot}63^{FGH}$	$-1{\cdot}00^{DE}$	$-1{\cdot}18^{F}$	$-0{\cdot}53$
ST3+ST4	$0{\cdot}154^{FG}$	$5{\cdot}11{\cdot}10^{3DEF}$	$27{\cdot}29^{DE}$	$33{\cdot}1^{FG}$	$0{\cdot}56^{ABCD}$	$0{\cdot}68^{CD}$	$0{\cdot}84^{A}$	$-0{\cdot}07^{CDEFG}$	$0{\cdot}78^{ABCD}$	$-0{\cdot}37^{DEF}$	$-0{\cdot}13$
ST3+ST5	$0{\cdot}158^{F}$	$5{\cdot}25{\cdot}10^{3DEF}$	$32{\cdot}04^{BCDE}$	$68{\cdot}9^{DEFG}$	$0{\cdot}70^{ABC}$	$1{\cdot}91^{ABC}$	$0{\cdot}89^{A}$	$-0{\cdot}82^{GHI}$	$-0{\cdot}99^{DE}$	$0{\cdot}00^{CDEF}$	$-0{\cdot}98$
ST5+ST2	$0{\cdot}186^{CD}$	$5{\cdot}51{\cdot}10^{3BCD}$	$47{\cdot}65^{A}$	$100{\cdot}1^{BCDE}$	$0{\cdot}90^{A}$	$2{\cdot}60^{A}$	$0{\cdot}34^{ABC}$	$-1{\cdot}47^{HI}$	$2{\cdot}08^{A}$	$0{\cdot}28^{BCDE}$	$0{\cdot}20$
ST5+ST4	$0{\cdot}190^{CD}$	$5{\cdot}27{\cdot}10^{3DEF}$	$38{\cdot}47^{ABC}$	$12{\cdot}8^{G}$	$0{\cdot}72^{AB}$	$2{\cdot}40^{AB}$	$1{\cdot}06^{A}$	$-1{\cdot}67^{I}$	$0{\cdot}99^{ABCD}$	$-0{\cdot}59^{DEF}$	$-0{\cdot}22$
ST4+ST2	$0{\cdot}184^{CD}$	$5{\cdot}86{\cdot}10^{3ABCD}$	$35{\cdot}87^{BCD}$	$78{\cdot}3^{CDEF}$	$0{\cdot}03^{ABCDE}$	$-0{\cdot}22^{DE}$	$-1{\cdot}08^{DE}$	$0{\cdot}40^{CD}$	$-0{\cdot}19^{BCD}$	$0{\cdot}60^{ABCD}$	$-0{\cdot}38$
Pooled Std.	0·008	425	4·95	26·45	0·65	0·81	0·52	0·44	0·95	0·64	0·75

ST1 = *Strep. thermophilus* CHCC-5581
ST2 = *Strep. thermophilus* CHCC-2389
ST3 = *Strep. thermophilus* CHCC-3021
ST4 = *Strep. thermophilus* CHCC-4239
ST5 = *Strep. thermophilus* CHCC-5086

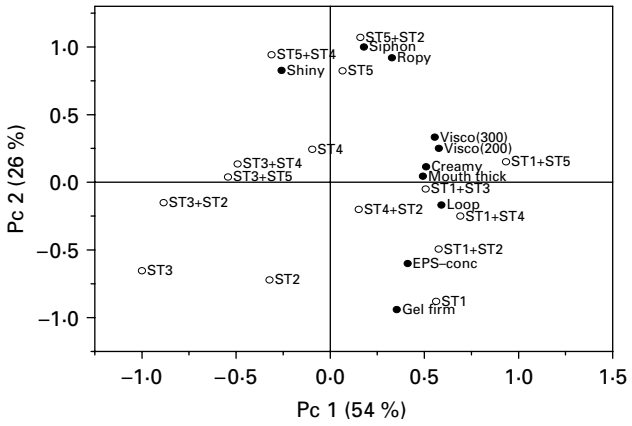


Fig. 1. Bi-plot of principal components (Pc) 1 and 2 from Principal Component Analysis on level corrected sensory data, rheological variables and exopolysaccharide (EPS) concentration of 15 stirred yoghurts produced with different EPS-producing cultures. Data are standardized and averaged over two replicates. The two principal components explain a total of 80% of the variation in the data set.

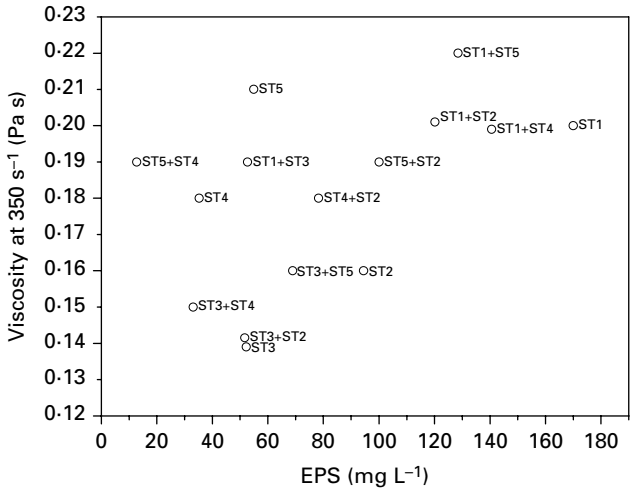


Fig. 2. Apparent viscosity (measured at shear rate 300 s^{-1}) as a function of exopolysaccharide (EPS) concentration for 15 stirred yoghurts produced with different EPS-producing cultures.

correlated with mouth thickness and viscosity (Pc 1) and in Pc 2 it was positively correlated with gel firmness. Figure 2 shows the apparent viscosity (at shear rate 300 s^{-1}) as a function of EPS concentration. Yoghurts produced with ST1 had the highest EPS concentrations followed by the mixtures of ST1 with ST4, ST5 or ST2 respectively. Medium concentrations of EPS were produced by ST2 and in its mixtures with ST5 or ST4. The single strains ST4, ST3 and ST5 and their mixtures gave low EPS concentrations.

Along the third Pc (not shown) creaminess, ropiness and mouth thickness was negatively correlated with viscosity and extensional viscosity (siphon tests) indicating that there were elements in these sensory properties that were not measured by the rheological analyses.

The highest mouth thickness, creaminess and viscosity was found in yoghurts fermented with the mixed culture of ST1+ST5 (Fig. 1). Such yoghurts obtained a significantly higher mouth thickness score (1·75) and viscosity than either of the constituting strains alone (mouth thickness respectively −0·34 and −0·48), showing that a positive interaction occurred between these strains. A positive interaction was also present regarding mouth thickness in yoghurts produced with the mixed culture ST1+ST3; this culture gave a significantly higher mouth thickness than the single strains alone. Also the mixed cultures ST1+ST4 and ST1+ST2 produced very mouth thick, creamy and viscous products. Another positive interaction (though not statistically significant) was observed for the mixture of ST2+ST4, which gave more mouth thick and viscous yoghurts than the single strains did. Yoghurts produced with especially ST3 and ST3+ST2 were thin and exhibited low creaminess and mouth thickness. The single strain ST3 also gave low ropiness, but ropiness was enhanced when ST3 was mixed with any other strain. ST5 and its mixture with ST4 as well as the mixture ST5+ST2 impacted high shininess and ropiness to the yoghurts. Both ST1 as a single strain as well as in all its mixtures, and the single strains ST3 and ST2 increased gel firmness and reduced ropiness.

The study did not include non-EPS-producing cultures, but previous studies including such cultures showed that EPS-free yoghurts had significantly higher syneresis and gel firmness as well as significantly lower mouth thickness, creaminess and viscosity than any EPS-containing yoghurt (Folkenberg et al. 2005).

Set yoghurts

In general, fewer significant differences among the yoghurt cultures were found for the set type yoghurts than for the stirred yoghurts. This suggests that the choice of fermentation culture is not as crucial for set yoghurt production as for production of stirred yoghurt. Gel firmness, which is the main texture property of set yoghurt, did not differ significantly among the set yoghurts produced with different cultures. This can be explained by the fact that gel firmness in set yoghurt is mainly governed by the protein network and not by EPS produced by the culture (van Marle & Zoon, 1995).

Capsule test

The capsule tests performed by CLSM showed that many of the investigated strains produced CPS. Figure 3 shows, as an example, the CPS produced by the strain ST4; the CPS appears as dark zones surrounding the cells. The strains ST1, ST4 and ST5 produced numerous capsules and ST2 also produced some capsules, but fewer than the other strains. The CPS sizes for all strains were in the range 2–5 μm, which is in agreement with the sizes reported by Hassan et al. (1995) for *Strep. thermophilus*. No CPS production was detected for strain ST3.

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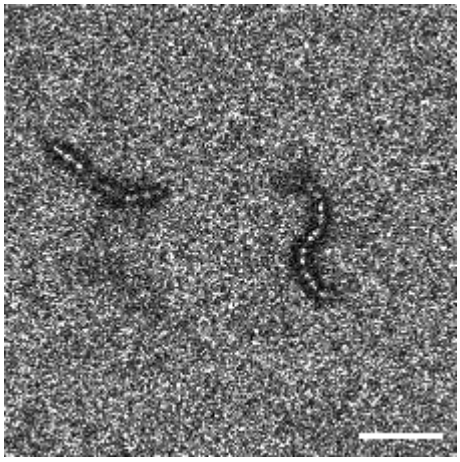


Fig. 3. Capsular polysaccharides (CPS) produced by the *Strep. thermophilus* strain CHCC-4239 (referred to as ST4 in current text) grown in milk and visualised by confocal laser scanning microscopy. The CPS appears as dark zones surrounding the chains of cells. The scale bar represents 10 μm.

Microstructure – CLSM images

When interpreting the images it must be kept in mind that only protein was visualised in the applied CLSM technique, and thus any other substances (e.g. EPS) appeared as black areas. It is thus possible that non-detected variations between the yoghurts existed. However, the protein images may provide indirect information about EPS distribution supported by the fact that the protein network structures observed differed significantly among the yoghurts even though only the fermentation culture was varied.

Figure 4 A+B show loading plot, respectively, bi-plot from PLS regression on all 256 mean angle values (MAs) versus level corrected sensory data for stirred yoghurts. It appears from Pc 1 in Fig. 4A that yoghurts characterized by the sensory descriptor gel firmness, and to a minor extent also mouth thickness, had higher MAs (i.e. more contrast complexity) at all scales compared with yoghurts characterized by the descriptors ropiness and shininess. The bi-plot (Fig. 4B) shows that yoghurt fermented with ST1+ST2 had the highest mean angles, and in general products containing one of the strains ST1, ST2 or ST3 had high contrast complexity. When viewing these images visually, protein networks with extensive connectivity between individual protein aggregates and small pores were seen. Yoghurts produced with ST5 or ST5+ST4 had the lowest contrast complexity, and in general yoghurts containing one of the strains ST5 or ST4 had low contrast complexity. Visual inspection of these images showed that the pores were large and the protein aggregates were much smaller than the pores resulting in a weak protein network with poor contact between the individual protein aggregates.

Thus, the results indicate that the CLSM images of the protein networks could be related to the gel firmness and ropiness. Figure 5 shows CLSM images of yoghurts produced with ST1+ST2, ST4+ST5 (the two extremes regarding protein structure) and ST1+ST5 (the yoghurt with the highest viscosity, mouth thickness and creaminess). The pH values of the yoghurts measured at the day the CLSM was carried out were comparable (respectively 4·37, 4·40 and 4·36). A dense protein network (Fig. 5A) gave rise to high gel firmness, whereas a loose protein network (Fig. 5C) resulted in low gel firmness. This is in accordance with van Marle & Zoon (1995) who found that protein-protein interactions, rather than EPS, governed the rheological behaviour measured at small deformations (relating to gel firmness). The large pores observed in some products (Fig. 5C) may contain ropy EPS that is not fixed by the protein network, and we believe that this structure results in the high ropiness and high serum ropiness perceived by the sensory panel. The yoghurts that have the highest creaminess, mouth thickness and viscosity (Fig. 5B) have microstructure structures with strong protein networks with good contact between the individual protein aggregates but with significantly larger pores than the products with the highest gel firmness. The mode of action by which ST1 reduced ropiness could be by fixating the EPS inside the pores by a strong protein network. The results clearly suggest that the pores and their content are important for the perceived yoghurt texture and that the distribution of the two main texture modelling ingredients, protein and EPS, is central for understanding the texture of fermented milk products.

The AMT method was not suitable for differentiating between the set yoghurts. This could be due to the large in-homogeneity in these products, compared with stirred yoghurts, which caused such high variation within images of the same yoghurt (or replicate fermentations with the same culture) that the differences between the cultures lost significance. Alternatively, the differences between the yoghurts may have been impacted by the stirring procedure.

Discussion

Inspection of data showed that ST1 and ST2 as single-ST-cultures had rather similar properties; and this result is in accordance with the fact that both ST1 and ST2 tended to positively interact with ST4 and ST5. The positive textural interactions between ST1 and several of the other strains, and to a minor extent between ST2 and other strains, could indicate that these produce EPS of a different nature from the others, and that the different types of EPS complement each other beneficially as regards the resulting yoghurt texture. In a previous study, the distribution of protein and EPS in set yoghurts produced with the cultures ST1, ST2, ST3 and ST4 were evaluated by CLSM. Staining with fluorescence marked lectins was applied to directly visualise the EPS (Hassan et al. 2002; Folkenberg et al. 2005). In yoghurts fermented with ST4 or ST3, the EPS were well

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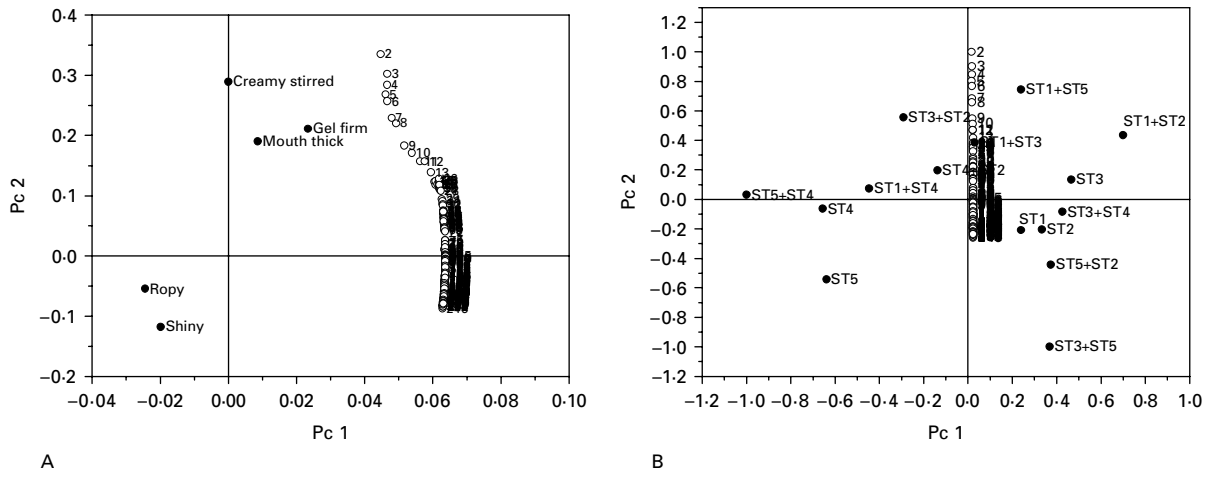


Fig. 4. Loading plot (A), respectively Bi-plot, (B) from Partial Least Squares regression on all 257 mean angles from Angular Measure Technique image analysis on confocal laser scanning microscopy images of stirred yoghurts (X-matrix) versus level corrected sensory data on stirred yoghurt (Y-matrix) (the numbers from 2 to 257 refer to the mean angle values for the respective S-values. Data are standardized and averaged over replicates.

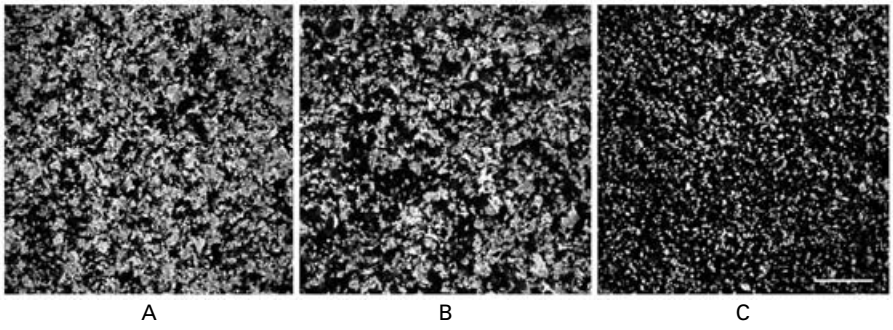


Fig. 5. Confocal laser scanning microscopy images of stirred yoghurts fermented with the cultures ST1+ST2 (image A), ST1+ST5 (image B) and ST4+ST5 (image C). A and C are the two opposite extremes as analysed by Angular Measure Technique image analysis and B is the yoghurts with the highest creaminess and viscosity. The scale bar represents 150 μm.

distributed in the product, primarily placed *around* the pores in the protein network, whereas the EPS were located *inside* the pores i.e. in the serum phase in yoghurts fermented with ST1 or ST2. We believe that in one type of microstructure observed, the EPS interact positively with the protein network, possibly due to charge interactions, whereas in the other type of microstructure observed there appears to be a lack of interaction, resulting in the EPS being placed in the pores of the network. The strain ST5 was not included in these analyses, but other characteristics of this strain indicate that it resembles ST4. In the present study, especially the strains ST5, and ST4, and to some extent ST3, seemed to benefit from being mixed with ST1 or ST2. This may be explained by a positive interaction of combining one type EPS that associates with the protein with another type of EPS which exits in the serum pores and thereby can be expected to greatly increase the serum viscosity. These results seem in agreement with Bouzar et al. (1997) who found positive texture effects when using mixed EPS-producing cultures.

The EPS-concentration affected the functional properties of the yoghurts and some positive correlations between high EPS-concentration and high creaminess and viscosity etc. were seen. However, several yoghurts did not follow this tendency. For instance, the most creamy and viscous product ST1+ST5 tended to have lower EPS-concentration than ST1 alone. A very low EPS concentration but a surprisingly high viscosity were observed for ST5+ST4, whereas the least creamy and viscous yoghurt (produced with ST3) did not have the lowest EPS-concentration. The mixed culture ST1+ST3 produced as little EPS as ST3 did alone, but the viscosity of the mixed culture was significantly higher (Fig. 2). Interestingly, mouth thickness correlated better with EPS concentration than ropiness did. Thus this study seems to confirm earlier findings that the EPS-protein interplay rather than the EPS concentration govern the functional properties (Marshall & Rawson, 1999).

Capsular polysaccharide (CPS) producing strains were found to contribute to high viscosity, mouth thickness and

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creaminess. Furthermore it appears that mixed cultures of two distinct CPS-producing strains give the highest viscosity and thus the ability to produce CPS seems to be important for the texture performance in yoghurts. However, as tendencies to interactions are found between two CPS-producing strains as well as between strains with high- and no-CPS-production, the ability to produce CPS does not seem to provide an explanation for the interaction tendencies.

The siphon test, which gives an empiric measure of the extensional viscosity, provided a very good estimation of the ropiness in these yoghurts.

In conclusion, the underlying properties of texture seem complex and multidimensional, and many characteristics must be present to achieve an optimal texture. From the results described in the present paper, as well as earlier results, we propose the following list of characteristics which seem to increase the mouth thickness, creaminess and viscosity in stirred yoghurts exploiting exopolysaccharides produced by the starter cultures for stabilization:

1. A protein network with good connectivity between the individual protein aggregates (a strong net work, but not too dense).
2. Medium size pores containing EPS. The pores must be large enough to contain a certain amount of free 'ropy' EPS, but small enough to ensure that the content of the pores are fixed within the protein network; otherwise very high sensory ropiness and syneresis of (ropy) serum occurs.
3. Capsular polysaccharides (CPS) are beneficial.
4. Different types of EPS-protein distribution; EPS present both in serum pores and in association with protein.
5. EPS concentration; to some extent. At least some EPS must be present to achieve a good texture, but other factors seem more important than concentration per se.

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