Impact of starch and exopolysaccharide-producing lactic acid bacteria on the properties of set and stirred yoghurts

Marie-Claude Gentès[a,b,1], Sylvie L. Turgeon[a,*], Daniel St-Gelais[a,b]

[a] *Dairy Science and Technology Research Centre (STELA), Institute of Nutrition and Functional Foods (INAF), 2425 De Agriculture Street, Paul-Comtois Building, Room 1316, Université Laval, Quebec City, QC, Canada G1V 0A6*

[b] *Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant Boulevard West, Saint-Hyacinthe, QC, Canada J2S 8E3*

[*]Corresponding author: Sylvie L. Turgeon, Dairy Science and Technology Research Centre (STELA), Institute of Nutrition and Functional Foods (INAF), 2425 De Agriculture Street, Paul-Comtois Building, Room 1316, Université Laval, Quebec City, QC, Canada G1V 0A6. Tel: +1 418 656 2131, ext. 4970. Fax: +1 418 656 3353. E-mail address: sylvie.turgeon@fsaa.ulaval.ca.

[1]Present address: Parmalat Canada, Marieville, QC, Canada J3M 1E1.

E-mail addresses:

Daniel St-Gelais: daniel.st-gelais@agr.gc.ca

Marie-Claude Gentès: marie-claude_gentes@parmalat.ca

Abstract

The impact of exopolysaccharide (EPS)-producing lactic acid bacteria with well-known structures and starch (0.75%) on the rheological properties (apparent viscosity and elastic modulus) and physical properties (syneresis) of set and stirred yoghurts was studied. Three EPS-producing strains with different structural characteristics were studied: *Streptococcus thermophilus* ST1 (anionic, stiff and linear EPS), *Lactobacillus delbrueckii* subsp. *bulgaricus* LB1 (neutral, stiff and ramified EPS) and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB2 (neutral, flexible and highly ramified EPS). The presence of linear, stiff, and anionic EPS from ST1 increased the elastic modulus in all yoghurt conditions, possibly owing to electrostatic interactions with caseins. Higher viscosity values were obtained with stiff and linear or slightly branched EPS from the ST1 and LB1 for all yoghurt conditions. Starch addition increased the values of the rheological and physical properties of all stirred yoghurts probably due to the repulsion between proteins and polysaccharides favoring thermodynamical incompatibility.

1. Introduction

In Canada, modified starch is often used in yoghurt formulations as a stabilizer, to limit technological defects such as whey separation (syneresis) and variations in viscosity due to its low cost and diversity. The usage of exopolysaccharides (EPS)-producing lactic acid bacteria (*Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* subsp. *bulgaricus*) in yoghurt manufacture is common, too. Exopolysaccharides are naturally produced during the fermentation process. Thus, starters can then perform two functions: formation of the protein network, which is responsible for yoghurt texture during fermentation, and addition of functionality through the capacity of EPS to improve serum retention and modulate viscosity (Gentès, St-Gelais, & Turgeon, 2011, 2013). The ability of EPS to modulate the rheological properties of yoghurt is not completely related to their concentration but also to their structural characteristics such as charge, molecular weight, composition in monomers, degree of branching, backbone stiffness, and EPS interactions with proteins as observed in other studies (Faber, Zoon, Kamerling, & Vliegenthart, 1998; Gentès et al., 2011, 2013; Girard & Schaffer-Lequart, 2007a, b; Petry et al., 2003). To date, no scientific publication has studied the effect of the combination of starch and EPS-producing lactic acid bacteria in yoghurt. Olsen (2003) found that the non-optimal combination of pectin and EPS-producing lactic acid bacteria can lead to defects. However, the mechanism responsible is poorly understood and being essential to yoghurt development with desirable properties.

To mimic industrial conditions in the present study, set and stirred yoghurts were made at the pilot scale. Few authors have studied the impact of stirring (commonly reported in the literature as being done with a spoon) on the microstructure of fermented milk with EPS but without the presence of stabilizers (Hassan, Corredig, & Frank, 2002; Hassan, Ipsen, Janzen, & Qvist, 2003; Girard & Shaffer-Lequart, 2007a). Hassan and co-workers (2003) observed that stirring fermented milk did not homogeneously mix EPS within the protein network but instead promoted local EPS concentration. As the EPS structure was unknown, no structure–function relationship was established. Girard & Shaffer-Lequart (2007a) showed that mixing fermented milk with a spoon led to a more homogenous protein network for anionic EPS in comparison with neutral EPS. This effect was attributed to associative electrostatic interactions between caseins and anionic EPS. However, no studies have reported the effect of stirring by using conditions closer to industrial process (using smoothing devices in a pilot plant) on the resulting rheological and physical properties of yoghurt fermented with EPS-producing lactic acid bacteria with well-known structures.

The aim of this work was to study the impact of EPS-producing lactic acid bacteria and starch with several reported structural characteristics (charge, degree of branching, and stiffness) on the rheological and physical properties (apparent viscosity, syneresis, and elastic modulus) of set and stirred low-fat yoghurts made on a pilot scale. The effect of a short storage period (8 days at 4 °C) was also studied.

2. Materials and methods

2.1. Materials

Pasteurized skim milk (Natrel, St-Laurent, QC, Canada), whey protein isolate (82% whey proteins, 98% dry matter; Davisco Foods International, Le Sueur, MN, USA), skim milk powder (low-heat, spray-drying process, 29% caseins, 5.4% whey proteins, 98% dry matter; René Rivet Inc., Terrebonne, QC, Canada), lactose (98% sugar; Saputo Dairy Products, St-Léonard, QC, Canada), and modified starch from waxy maize (87% total carbohydrates, 91% dry matter; Thermtex, Henkel, Mississauga, ON, Canada) were used to make yoghurt. For each batch, the composition of pasteurized skim milk (Agropur cooperative, Longueuil, QC, Canada) was determined with a Fourier transform infrared analyzer (Model FT120; Foss North America, Eden Prairie, MN, USA). All previous ingredients were used to standardize the yoghurt composition of 14% dry matter, 4.0% total protein, 3.0% caseins, and 0.75% whey proteins, with or without starch (0.75%).

2.2. Preparation of bacterial strains and starters

Three EPS-producing lactic acid bacteria were used in this study: *Streptococcus thermophilus* NIZO2104 (ST1), *Lactobacillus delbrueckii* subsp.*bulgaricus* DGCC291 (LB1) and *Lactobacillus delbrueckii* subsp.*bulgaricus* NCIMB702074 (LB2). Each EPS-producing lactic acid bacteria was mixed with its complementary control strain to constitute a starter for yoghurt production: *Streptococcus thermophilus* HC15 or *Lactobacillus delbrueckii* subsp. *bulgaricus* 210R. HC15 and 210R were also combined together to constitute the control starter. EPS structural characteristics were presented in Table 1. Stock cultures of single EPS-producing lactic acid bacteria and control strains were stored at −80 °C in 20% (w/w) reconstituted skim milk (RSM) sterilized at 110 °C for 10 min. The RSM was made from skim milk powder rehydrated in distilled water and supplemented with 5% (w/w) sucrose (Fisher Scientific, Nepean, ON, Canada). As culture medium, a 12% (w/w) RSM was prepared by dissolving skim milk powder in distilled water, stirring at room temperature for 2 h, and sterilizing at 110 °C for 10 min in an autoclave. The sterilized RSM was stored at 4 °C until use. Active strains were obtained by inoculating RSM (100 mL) at 12% (v/w) with stock culture and incubating at 37 °C for 16 h. The strains were subcultured at 3% (v/w) for 3 h for LB2, 3.5 h for 210R (control), 4 h for HC15 (control), 4.5 h for LB1, and 6 h for ST1 at 42 °C in 1.7 kg of RSM that had been heat-treated (90 °C for1 min) beforehand with an automatic steam-controlled water bath designed for dairy starter preparation (Laboratorium Wiesby GmbH & Co., Niebüll, Germany). Fermentation was performed in an incubator (CS-20;Coldstream Drive, Jordan Valley, IL, USA) until the pH reached 5.2 for streptococci and 4.8 for lactobacilli, and then the active strains were rapidly cooled to 4 °C in ice. A population of more than 1×10^8 CFU mL^{-1} was reached for all strains. For the ST1 strain, another subculture at 3% (v/v) and fermented at 42 °C for 6 h was necessary to reach a population of 1×10^8 CFU mL^{-1}. All active strains were stored overnight at 4 °C before use. On the production day, the active strains were mixed together to obtain each starter combination (one streptococci and one lactobacilli). Depending on the population of each active strain, the appropriate quantities were added to obtain an initial population of 2×10^7 CFU mL^{-1} with a ratio of 50:50 for the control, LB1, and LB2 strain combinations but 65:35 for the ST1combination. These ratios had been previously determined to provide the same acidification time (pH 4.6 after 4 h at 42 °C) for all strain combinations. Populations of active strains during yoghurt production and during storage were enumerated on M17 medium (Oxoid; VWR, Montreal, QC, Canada) for streptococci and on acidified MRS medium (Difco; VWR) for lactobacilli under anaerobic conditions.

2.3. Manufacture of yoghurts

Set and stirred yoghurts were made at the pilot scale. Eight different yoghurts were prepared, with or without starch (0% and 0.75% (w/w)) and four starters. Set and stirred yoghurts were made with the same recipe. Yoghurt made with starch (total of 116 kg batch) was prepared by mixing 2.78 kg of RSM, 0.2 kg of whey protein isolate, 100 kg of skim milk, 3.89 kg of lactose and 0.95 kg of starch. For yoghurt without starch, all ingredients were added at the same level except that the lactose quantity was added at 4.77 kg to standardize the total solid content. Solid ingredients were added to liquids with a centrifuge pump (25,000 L h^{-1}) and mixed for 5 min. Batches were homogenized at 55 °C in two stages, 3.44 MPa and 10.34 MPa (Model SHL 20homogenizer; Alpha Laval, Scarborough, ON, Canada), followed by heat treatment of 90 °C for 1 min (Type C3-SR plate pasteurizer, 2005, capacity of 2000 L h^{-1}; designed for Tetra-Pak by Alpha Laval, Scarborough, ON, Canada) and cooled to 42 °C with a cooling plate exchanger. The batch was split into four portions (18.6 kg each) and inoculated with the appropriate starter quantity: 404 g for HC15 (control), 994 g for ST1, 418 g for 210R (control), 415 g for LB1, and 369 g for LB2. Because the quantities of added starters differed due to the different bacterial population, RSM was added to reach a final weight of 1.4 kg for each condition. Each batch contained a final weight of 20 kg. After inoculation, 10 kg of the batch was placed into 175-mL plastic cups and incubated at 42 °C in an incubator (CS-20; Coldstream Drive, Jordan Valley, IL, USA) to produce set yoghurts. The resulting set yoghurts were rapidly transferred into a chamber at 4 °C. For stirred yoghurts, 10 kg of the batch was fermented directly in the 25 kg stainless steel container at 42 °C in a room incubator. Fermentation was stopped when the pH reached 4.6 ± 0.05. The resulting set yoghurt was gently stirred 10 times with a stainless steel utensil, cooled to 20 ± 2 °C using a mobile plate exchanger system (Type P30A, PR-16, WB-B series; Alpha Laval, Scarborough, ON, Canada), and smoothed at 0.27 MPa with a screw pump (Allweiller, NetzschAG, 0–100 L h^{-1}; Radolfzell, Germany). The stirred yoghurt was then poured into 175-mL plastic cups and rapidly transferred to storage at 4 °C. The changes in pH and rheological and physical properties were measured after 2 and 8 days of storage at 4 °C.

2.4. Analytical methods

Lactic acid production (difference between final titrable acidity and initial titrable acidity), pH, dry matter and ash content of yoghurt were measured by the official standard methods (AOAC, 2000). Total protein, noncasein nitrogen, and nonprotein nitrogen were measured by using the macro-Kjeldahl method (St-Gelais, Roy, & Audet, 1998). The noncasein nitrogen content in the unheated milk mixture was obtained by casein precipitation at pH 4.6 with H_2SO_4 (0.02 N). The acid solution was filtered (Whatman paper no. 40), and the filtrate was analyzed. The nonprotein content was obtained by protein precipitation with 12% trichloroacetic acid (w/w). The sample was filtered (Whatman paper no. 40) and analyzed (St-Gelais et al. 1998). The casein and whey protein contents were calculated by difference. A nitrogen conversion factor of 6.38 was used.

2.5. Rheological and physical property measurements

The rheological properties of yoghurts were measured with a dynamic stress rheometer using a bob and a cup (Model SR-2000; TA Instruments, New Castle, DE, USA). To transfer stirred and set yoghurts from the plastic cups in the rheometer geometry with minimal disruption of the gel, samples were carefully taken from the plastic pots with a homemade stainless steel cylinder (length of 0.123 m and internal diameter of 0.018 m) and poured carefully in the rheometer. The bob is slowly lowered to the set gap. The viscosity was measured by a steady stress sweep test with a shear stress of 1.0-100 Pa. Apparent viscosity at 10 and 100 s^{-1} was calculated according to the power law model (Everett & McLoed, 2005). The elastic modulus (G′) and viscous modulus (G″) were measured at 0.1 Pa (stirred yoghurt) and 1 Pa (set yoghurt), in the linear region of viscoelasticity for each yoghurt, as a function of a frequency range of 0.1 to 10 Hz with a dynamic stress rheometer using a bob (diameter of 29.5 mm, length of 44.25 mm) and a cup (internal diameter of 32 mm) (Model SR-2000; TA Instruments, New Castle, DE, USA). Syneresis was measured by centrifugation technique (Everett & McLeod, 2005). Samples (25 g) were directly taken from the plastic cups with the homemade stainless cylinder to minimise disruption of gel and were centrifuged at 1,900 x *g* for 20 min at 4 °C. The clear supernatant was poured off, weighed and recorded as syneresis (%). All measurements were performed in duplicate at 4 °C after 1 (for viscosity only), 2 and 8 days of storage.

2.6. Microscopy

The set and stirred yoghurts were observed by confocal laser scanning microscopy (CLSM) operating in fluorescence mode with a He/Ne laser (Nikon TE-2000E Eclipse; Nikon, Mississauga, ON, Canada). After inoculation, the set yoghurts milks (10 mL) were transferred into 50-mL sterile tubes and stained with 30 µL of acridine orange (protein dye) at 0.2% (w/w) (Sigma-Aldrich, Oakville, ON, Canada) according to the method of Lee & Lucey (2004). The samples were gently mixed by inversion five times. Then, 200-µL samples were transferred into microscope wells (VWR), the cover slips were fixed with Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, MI, USA), and slides were put into petri dishes covered with parafilm to prevent dehydration. All samples were incubated at 42 °C in the same incubator used for yoghurt manufacture. When the pH reached 4.6, the samples were stored at 4 °C for 48 h. For the stirred yoghurts, 10-mL samples were taken after the smoothing process, transferred into 50-mL sterile tubes, and stained and mixed as described above. Then, 200-µL samples were transferred into microscope wells and treated exactly as described above for the set yoghurts. The microscope wells containing the stirred yoghurts were stored at 4 °C for 48 h before observation. The samples were observed at an excitation wavelength of 488 nm with a water-immersion 60×objective lens (numerical aperture of 1.4) at a depth of 10 to 20 µm. The emission of fluorescence was recorded between 525 and 555 nm. Three pictures were taken for each sample and only representative images are presented.

2.7. Statistical methods

A split-split-split-plot design was used to study the bacterial population, pH, and rheological and physical properties during storage of set and stirred yoghurts made with or without starch and fermented with EPS-producing lactic acid bacteria with well-known structures. Set and stirred yoghurts are obviously very different in term of structure. The statistical analysis revealed that rheological and physical properties variables studied were always significant ($p < 0.005$) and masks other relevant differences. The analysis was therefore realized for set and stirred yoghurt independently throughout a split-split-plot design for pH, viscosity, G' and syneresis. Significant differences were tested at $p < 0.05$. Statistical analysis was carried out with the general linear models procedure of the SAS software program (Version 9.1.3, 2003; SAS Institute, Cary, NC, USA). The experiments were performed in triplicate.

3. Results

3.1. Composition and fermentation

The composition of all yoghurts was not significantly different: 3.02 ± 0.05% caseins, 0.797 ± 0.003% whey proteins, 3.95 ± 0.07% total protein, and 13.77 ± 0.07% dry matter. The initial pH of all blends after inoculation was not significantly different: pH 6.50 ± 0.04. The initial bacterial population was $3.1 \pm 0.1 \times 10^7$ CFU mL^{-1} with a streptococci-to-lactobacilli ratio of 57 ± 3 for all starters except for ST1, for which the initial bacterial population and the streptococci-to-lactobacilli ratio were $1.88 \pm 0.09 \times 10^7$ CFU $mL^{-1}$and 44 ± 3, respectively. At the end of fermentation (181 ± 1 min), all yoghurt types had a final pH of 4.54 ± 0.03 and a lactic acid production of 0.52 ± 0.2%. The biological population was statistically similar for all yoghurts at the end of fermentation: $4.90 \pm 0.06 \times 10^8$ CFU mL^{-1} and $3.25 \pm 0.03 \times 10^8$ CFU mL^{-1} for streptococci and for lactobacilli, respectively. Streptococci and lactobacilli populations were significantly affected by yoghurt type and storage time, but data are not shown, because the difference was small (less than 1.6×10^0 CFU mL^{-1}).

The change in pH at 4 °C was significantly influenced by starter and storage time for both yoghurt types ($p < 0.0009$) (Fig. 1). A significant interaction was observed for set yoghurt between starch and storage time ($p = 0.003$). The set yoghurts without starch had similar pH values among all starters except LB1, which had a higher pH value after 2 days. For the set yoghurts with starch, those fermented with the control and LB1 starters had significantly higher pH values than those fermented with the other starters after both 2 and 8 days. For all starters, the pH values decreased significantly during the storage period for the set yoghurts with starch. For the stirred yoghurts pH values were different according to starter, LB1 and LB2 having higher pH values overall but all strains showed a pH reduction overtime.

3.2. Rheological and physical properties of yoghurt during storage

3.2.1. Apparent viscosities

The apparent viscosity of the set yoghurt was significantly affected by starter ($p = 0.001$), storage period ($p = 0.0185$) and their interactions ($p = 0.00034$) while for stirred yoghurt a starch*starter and a starch*starter*storage period significant interactions were observed

(Fig. 2). No significant interactions were found. All EPS-producing starters resulted in set yoghurts with a higher viscosity than was obtained with the control starter (Fig. 2a). The apparent viscosity values of the set yoghurts fermented with the control, LB2, and ST1 starters were not significantly affected by the addition of starch. However, adding starch significantly increased the apparent viscosity value of the set yoghurt fermented with the LB1 starter. The apparent viscosity values increased slightly upon storage for all starters. The apparent viscosities were significantly higher for the ST1 starter in comparison with the control and LB2 starters.

Smoothing the yoghurts led to different viscosity profiles depending on the starter, starch addition, and storage period in comparison with the set yoghurts (Fig. 2b). The stirred yoghurts had a significantly lower apparent viscosity (2.88 ± 0.06 Pa·s) than the set yoghurts did (4.67 ± 0.06 Pa·s). The addition of starch led to an increase in apparent viscosity values for the stirred yoghurts. The apparent viscosities of the stirred yoghurts fermented with the control, LB2, and ST1 starters did not vary significantly or varied only slightly during the storage period, irrespective of starch addition. However, higher apparent viscosity values were measured during the storage period for the stirred yoghurt fermented with the LB1 starter, regardless of starch addition. As generally observed in set yoghurt, the stirred yoghurt fermented with the LB1 starter had the significantly highest apparent viscosity value.

3.2.2. Elastic modulus

The elastic modulus (G′) at 1 Hz for the yoghurts is presented in Table 2. The average of the G′ values for the stirred yoghurts with and without starch for all starter used (75.39 ± 8.33 Pa) was significantly lower than the average for the set yoghurts (366.16 ± 8.72 Pa). A linear relationship between the G′ and (log) frequency was observed (Supplementary Fig. S1). The G″ values (data not shown) were lower than the G′ values for all conditions, indicating the elastic or solid-like character of the gels. The G′ of set yoghurt was significantly affected by starter and storage period (Supplementary Fig. S1). The G′ values were significantly higher for the set yoghurts fermented with the ST1 and LB2 starters. The smoothing process had a significant impact on the G′ values and a double interaction between starter and starch could be observed (p = 0.0001). Without starch, G′ values were low and starch addition generally increased G′ values. The yoghurt made with starch and fermented with the ST1 starter showed the highest G′ value.

3.2.3. Syneresis

The syneresis of the set and stirred yoghurts was significantly affected by starter ($p < 0.0001$) while starch addition influenced stirred yoghurt only ($p < 0.01$) (Fig. 3). No significant effect of storage period was observed. A significant interaction between starter and starch addition was observed for set yoghurt. The set yoghurts fermented with the LB1 starter had significantly lower syneresis values, irrespective of starch addition as compared to the other starters for which starch addition favors lower syneresis. In contrast, this behaviour was no longer observed in stirred yoghurt with LB1 starter.

However, adding starch to the stirred yoghurt led to a significant decrease in syneresis, from 18 to 9% ± 0.5 for most conditions.

4. Discussion

4.1. Effect of starch and EPS in set yoghurt

The rheological properties (apparent viscosity and elastic modulus) and physical properties (syneresis) of the set yoghurts are generally governed by the protein network. The presence of EPS-producing lactic acid bacteria made an additional contribution to the rheological and physical properties but at different levels depending on their structural characteristics. The presence of the anionic, linear and stiff EPS from ST1 starter increased the apparent viscosity and the elastic modulus values of yoghurt without starch compared to the other starters as shown previously with fermented milk (Gentès et al., 2011) and dairy model system (Gentès et al., 2013). The effect on apparent viscosity was attributed to the stiffness and the linearity of the EPS resulting in a larger radius of the volume correlated with an increase in viscosity (Whistler & BeMiller, 1997). This may reinforce the protein network as observed by Laneuville & Turgeon (2014). However, these types of interactions between caseins and anionic EPS from the ST1 starter may have had a limited effect on serum retention. The electrostatic interactions between anionic EPS and caseins might hinder protein–water and EPS–water interactions, leading to a protein network with a lower ability to retain serum.

The presence of the neutral, stiff, and slightly branched EPS from the LB1 starter had a significant positive impact on apparent viscosity value and serum retention (low syneresis in comparison to the control starter). These results were in accordance with those obtained by Gentès et al. (2011, 2013). The EPS from the LB1, LB2 and ST1 starters had similar molecular weights (Gentès et al., 2013). The non-contribution of the EPS from the LB2 starter to viscosity and serum retention, in comparison with the EPS from the LB1 and ST1 starters, may have been due to the high level of branching and the flexibility of its EPS backbone, causing a smaller radius of volume. Van den Berg et al. (1995) showed that neutral EPS dissolve easily in the serum because they interact less with the positively charged caseins than anionic EPS do, and thus neutral EPS cause less syneresis. This effect was observed in the present study for the neutral EPS from the LB1 starter, which retained more serum than the anionic EPS from the ST1 starter.

The increase of the elastic modulus value of the yoghurt with starch, in the presence of the EPS from the LB2 starter was unexpected, since this type of EPS was previously found to behave like the control starter when used in fermented milk (Gentès et al., 2011) and a dairy model system (Gentès et al., 2013). The different compositions (casein-to-whey-protein ratio and dry matter) of fermented milk (Gentès et al., 2011), dairy model system (Gentès et al., 2013), and yoghurt (this study) might also contribute to differences in EPS functionality. In a dairy model system with 3% caseins, the rheological properties were driven mainly by the protein network, because EPS functionality was no longer observable for a dairy model system with 2% caseins (Gentès et al., 2013). Other researchers have underlined the importance of the composition (casein-to-whey-protein ratio and dry matter) of fermented milk for EPS functionality (Amatayakul, Halmos, Sherkat, & Shah, 2006; Amatayakul, Sherkat, & Shah, 2006). However, given that the structures of the EPS used by these authors were unknown, no EPS structure–function relationship could be established.

Using starch in combination with the control starter or the EPS-producing lactic acid bacteria had few effects on the rheological and physical properties of set yoghurt, as observed in a previous study using a dairy model system (Gentès, 2011). These effects can be due to some non-specific repulsive interactions, related to the excluded volume between these two biopolymers, resulting in segregative conditions as observed by others (Alloncle & Doublier, 1991). In comparison to other polysaccharides, including EPS, the functionality of starch with regard to rheological and physical properties cannot be explained by the radius of volume, due to its round shape structure. Starch structure is highly organized in a granule (Appelqvist & Debet, 1997). Functional properties of starch granules depend on the gelatinization process. During heat treatment, starch granules are progressively dissolved in aqueous solutions allowing hydration and swelling. Hydroxyl groups of amylose and amylopectin bind water and thus, increasing the viscosity (Eliasson, 2004). Consequently, the competition of other molecules such as proteins and EPS may affect the swelling process of starch granules and alter their functional properties, for example, the reduction of serum retention as observed in this study. Differences in gel pH should also be considered as a factor of influence on gel properties and syneresis. However, results obtained in this study could not be directly related to these properties, as an example for control yoghurt there was an increase in pH when starch was added while viscosity (Fig. 2) and G’ (Table 2) remained constant.

4.2. Effect of starch and EPS in stirred yoghurt

The smoothing process modified the rheological and physical properties of the yoghurt. No specific interactions between EPS-producing lactic acid bacteria and starch seemed to occur, because EPS functionality remained, and the presence of starch had an additional effect on the rheological and physical properties. Some authors postulated that the synergistic interaction between starch and polysaccharides may be attributed to phase separation (Alloncle & Doublier, 1991). Self-aggregation of starch granules due to a depletion flocculation mechanism has been suggested to explain the synergistic effect of starch and xanthan (Abdulmola, Hember, Richardson, & Morris, 1996). EPS from the LB1 starter had the highest ability to retain serum in set yoghurt but lost this property in stirred yoghurt. Hassan et al. (2003) observed that stirring with a spoon led to a more homogenous protein network with smaller pore sizes in comparison with set fermented milk with EPS. However, the structure of the EPS in their study was unknown. Therefore, the smoothing process might have broken the original web-like EPS structure that was possibly responsible for the enhancement of serum retention. This underlines the effect of shear on EPS functionality.

Starch increasing the apparent viscosity and serum retention of the stirred yoghurt, was also observed by Williams, Glagovskaia, & Augustin (2003). Given that the ability of starch to increase rheological and physical properties is a function of the swelling process (Oh et al., 2007), the less restricted volume seemed to have contributed to the functionality of starch. The optimal swelling of granules might have limited local serum mobility (aqueous phase), causing the enhanced apparent viscosity and elastic modulus and greater serum retention observed in this study.

It is known that the smoothing process breaks the initial protein network, causing a significant impact on the rheological and physical properties of yoghurt. Observations of the microstructure by CLSM (Supplementary Fig. S2) suggest that the presence of EPS-producing lactic acid bacteria and starch in the yoghurts may affect the gel microstructure. This is probably related to segregative conditions as observed previously in fermented milk with starch (Oh, Anema, Wong, Pinder, & Hemar, 2007) and in mixed solutions of EPS with caseins (Tuinier, ten Grotenhuis, Holt, Timmins, & de Kruif, 1999) and EPS with whey proteins (Tuinier, Dhont, & De Kruif, 2000). Many authors observed that EPS (with known and unknown structures) or starch is located in the pores of the protein network (Girard & Schaffer-Lequart, 2007a; Hassan et al., 2003; Kalab, Emmonds, & Sargand, 1975; Oh et al., 2007; Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004). Although the starch and EPS used in this study were not stained, we hypothesize that EPS and starch could both be located in the pores (black areas) of the protein network. Girard & Schaffer-Lequart (2007a) observed a more homogenous microstructure in stirred (with a spoon) fermented milk with anionic EPS. This effect was attributed to the electrostatic interactions between caseins and anionic EPS in comparison with neutral EPS. In the present study, the charge had no impact on the homogeneity of the stirred yoghurt microstructure. This lack of effect may be explained by the different stirring process, given that Girard & Schaffer-Lequart (2007a) used a spoon, and in the present study a screw pump and a constant pressure were used. After the smoothing process (with a screw pump), thermodynamic incompatibility probably caused by the repulsion between proteins and polysaccharides seemed to be favoured.

5. Conclusions

This study has shown the effect of starch and EPS-producing lactic acid bacteria with known structural characteristics on the rheological and physical properties of set and stirred yoghurts. The rheological and physical properties of those yoghurt types were driven mainly by the protein network as influenced by EPS and starch respectively. Linear, stiff, and anionic EPS possibly owing to electrostatic interactions with caseins was most influential on the elastic modulus of the set yoghurt, whereas stiff and linear or slightly branched EPS were found to have a larger impact in terms of increasing viscosity values. Starch addition had little or no effect on the rheological and physical properties of the set yoghurt. The smoothing process had a significant impact on the rheological and physical properties of the yoghurt. This study has shown that EPS-producing lactic acid bacteria with specific structural characteristics may be used in association with starch to modulate the rheological and physical properties of yoghurt, especially for the stirred type. This underlines the significant impact of shear on functionality of EPS, starch and their combination in stirred yoghurt and the need for further investigation to develop yoghurt with desired sensorial properties.

Acknowledgements

This research was funded jointly by the research programs of the Fonds québécois de la recherche sur la nature et les technologies, Novalait Inc., the Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec, and Agriculture and Agri-Food Canada. The lead author would like to thank the Canadian Dairy Commission, Novalait Inc., and the Fonds québécois de la recherche sur la nature et les technologies for her graduate scholarship. The authors would also like to thank Gaétan Bélanger, research assistant at Agriculture and Agri-Food Canada's Food Research and Development Centre, for his expertise and technical assistance in the production of yoghurts at the pilot scale.

Figure captions

Fig. Changes in the pH of set (a) and stirred (b) yoghurts fermented with EPS-producing lactic acid bacteria (control (■), LB1 (■), LB2 (□) and ST1 (■) as a function of storage period at 4 °C. Means with different letters differ significantly.

Fig. Apparent viscosity at 10 s^{-1} of set (a) and stirred (b) yoghurts fermented with EPS-producing lactic acid bacteria (control (■), LB1 (■), LB2 (□) and ST1 (■) as a function of storage period at 4 °C. Means with different letters differ significantly.

Fig. Syneresis after centrifugation at 210 × *g* of set and stirred yoghurts fermented with EPS-producing lactic acid bacteria (control (■), LB1 (■), LB2 (□) and ST1 (■) as a function of storage period at 4 °C. Means with different letters differ significantly.

.

.

References

Abdulmola, N.A., Hember, M.W.N., Richardson, R.K., & Morris, E.R. (1996).Effect of xanthan on the small-deformation rheology of crosslinked and uncrosslinked waxy maize starch. *Carbohydrate Polymers, 31*, 65–78.

Alloncle, M., & Doublier, J.-L. (1991). Viscoelastic properties of maize starch/hydrocolloid pastes and gels. *Food Hydrocolloids*, *5*, 455–467.

Amatayakul, T., Halmos, A.L., Sherkat, F., &Shah, N.P. (2006). Physical characteristics of yoghurts made using exopolysaccharide-producing starter cultures and varying casein to whey protein ratios. *International Dairy Journal*, *16*, 40–51.

Amatayakul, T., Sherkat, F., &Shah, N.P. (2006). Physical characteristics of set yoghurt made with altered casein to whey protein ratios and EPS-producing starter cultures at 9 and 14% total solids. *Food Hydrocolloids*, *20*, 314–324.

AOAC. (2000). *Dairy products*. In: W. Horowitz (Ed.), Official methods of analysis of AOAC international (17^{th} ed.). (pp. 69-82). Gaithersburg, MD, USA: AOAC International.

Appelqvist, I.A.M., & Debet, M.R.M. (1997). Starch-biopolymer interactions—A review. *Food Reviews International*, *13*, 163–224.

Eliasson, A.-C. (2004). *Starch in food: Structure, function and applications*. Cambridge, UK: Woodhead Publishing; Boca Raton, FL, USA: CRC Press.

Everett D.W., & McLeod R.E. (2005). Interactions of polysaccharide stabilisers with casein aggregates in stirred skim-milk yoghurt. *International Dairy Journal*, *15*, 1175-1183.

Faber, E.J., Zoon, P., Kamerling, J.P., & Vliegenthart, J.F.G. (1998). The exopolysaccharides produced by *Streptococcus thermophilus* Rs and Sts have the same repeating unit but differ in viscosity of their milk cultures. *Carbohydrate Research, 310*, 269–276.

Gentès, M.-C. (2011). *Compréhension du rôle structural d'exopolysacharides de bactéries lactiques dans des systèmes laitiers fermentés enrichis en amidon modifié*. PhD thesis, Université Laval. Quebec City, QC, Canada. Retrieved from http://theses.ulaval.ca/archimede/fichiers/25036/25036.html.

Gentès, M.-C., St-Gelais, D., & Turgeon, S.L. (2011). Gel formation and rheological properties of fermented milk with in situ exopolysaccharide production by lactic acid bacteria. *Dairy Science & Technology*, *91*, 645–661.

Gentès, M.-C., St-Gelais, D., & Turgeon, S.L. (2013). Exopolysaccharide–milk protein interactions in a dairy model system simulating yoghurt conditions. *Dairy Science &Technology*, *93*, 255–271.

Girard, M., & Schaffer-Lequart, C. (2007a). Gelation and resistance to shearing of fermented milk: Role of exopolysaccharides. *International Dairy Journal*,*17*, 666–673.

Girard, M., & Schaffer-Lequart, C. (2007b). Gelation of skim milk containing anionic exopolysaccharides and recovery of texture after shearing. *Food Hydrocolloids*, *21*, 1031–1040.

Hassan, A.N., Corredig, M., & Frank, J.F. (2002). Capsule formation by nonropy starter cultures affects the viscoelastic properties of yogurt during structure formation. *Journal of Dairy Science*, *85*, 716–720.

Hassan, A.N., Ipsen, R., Janzen, T., & Qvist, K.B. (2003). Microstructure and rheology of yogurt made with cultures differing only in their ability to produce exopolysaccharides. *Journal of Dairy Science*, *86*, 1632–1638.

Kalab, M.,Emmonds, D.B., & Sargand, A. G. (1975). Milk-gel structure. IV. Microstructure of yoghurts in relation to the presence of thickening agents. *Journal of Dairy Research*, *42*, 453–458.

Laneuville, S.I., & Turgeon, S.L. (2014). Microstructure and stability of skim milk acid gels containing an anionic bacterial exopolysaccharide and commercial polysaccharides. *International Dairy Journal*, *37*, 5–15.

Oh, H.E., Anema, S.G., Wong, M., Pinder, D.N., &Hemar, Y. (2007). Effect of potato starch addition on the acid gelation of milk. *International Dairy Journal*, *17*, 808–815.

Olsen S. (2003). Microstructure and rheological properties of yoghurt, In International Dairy Federation (Ed.), *Proceeding of IDF seminar on aroma and texture of fermented milk* (pp. 302-312). Brussels, Belgium: International Dairy Federation Special issue no. 301.

Petry, S., Furlan, S., Waghorne, E., Saulnier, L., Cerning, J., & Maguin, E. (2003). Comparison of the thickening properties of four *Lactobacillus delbrueckii* subsp. *bulgaricus* strains and physicochemical characterization of their exopolysaccharides. *FEMS Microbiology Letters*, *221*, 285–291.

Sandoval-Castilla, O., Lobato-Calleros, C., Aguirre-Mandujano, E., & Vernon-Carter, E.J. (2004). Microstructure and texture of yogurt as influenced by fat replacers. *International Dairy Journal*, *14*, 151–159.

St-Gelais D., Roy D., & Audet P. (1998). Manufacture and composition of low fat Cheddar cheese from milk enriched with different protein concentrate powders. *Food Research International*, *31*, 137-145.

Tuinier, R., Dhont, J. K. G., & De Kruif, C. G. (2000). Depletion-induced phase separation of aggregated whey protein colloids by an exocellular polysaccharide. *Langmuir*, *16*, 1497–1507.

Tuinier, R., ten Grotenhuis, E., Holt, C., Timmins, P.A., & de Kruif C.G. (1999). Depletion interaction of casein micelles and an exocellular polysaccharide. *Physical Review E (Statistical Physics, Plasmas, Fluids, and Related Interdisciplinary Topics)*, *60*, 848–856.

van den Berg, D.J.C., Robijn, G.W., Janssen, A.C., Giuseppin, M.L.F., Vreeker, R., Kamerling, J.P., Vliegenthart, J.F.G., Ledeboer, A.M., & Verrips, C.T. (1995). Production of a novel extracellular polysaccharide by *Lactobacillus sake* 0-1 and characterization of the polysaccharide. *Applied and Environmental Microbiology*, *61*, 2840–2844.

Whistler, R.L., & BeMiller, J.N. (1997). Polysaccharides. In The American Association of Cereal Chemists (Ed.), *Carbohydrate chemistry for food scientists* (pp. 63–89).St. Paul, MN, USA: Eagan Press.

Williams, R.P.W., Glagovskaia, O., & Augustin, M.A. (2003). Properties of stirred yogurts with added starch: Effects of alterations in fermentation conditions. *Australian Journal of Dairy Technolog*y, *58*, 228–232.

Table 1. Structural characteristics of EPS-producing lactic acid bacteria.

Strain		Structural Characteristics			
Complete name	Abbreviation	Sugar composition	Sugar ratio	Charge	Molecular weight (g·mol^{-1})
Streptococcus thermophilus NIZO2104	ST1	Galactose:Ribose: Glucose: *N*-acetyl[2]	2 : 1 : 1 : 1	Negative	0.9×10^6
Lactobacillus delbrueckii subsp. *bulgaricus* DGCC291	LB1	Galactose: Glucose	2 : 3	Neutral	1.4×10^6
Lactobacillus delbrueckii subsp. *bulgaricus* NCIMB702074	LB2	Galactose: Glucose	4 : 3	Neutral	1.8×10^6

Table adapted from Gentès et al. 2011. Each EPS-producing strain was mixed with its complementary control strain to constitute starter for yoghurt production: *Streptococcus thermophilus* HC15 or *Lactobacillus delbrueckii* subsp. *bulgaricus* 210R.

[1]Branching = linear (-), one branching (+), more than two branching (++).

[2]*N*-acetyl = *N*-acetyl-galactosamine plus another monomer: 6-*O*-(3′,9′-dideoxy-D-*threo*-D-*altro*-nononic acid-2′-yl)-α-D-glucopyranose.

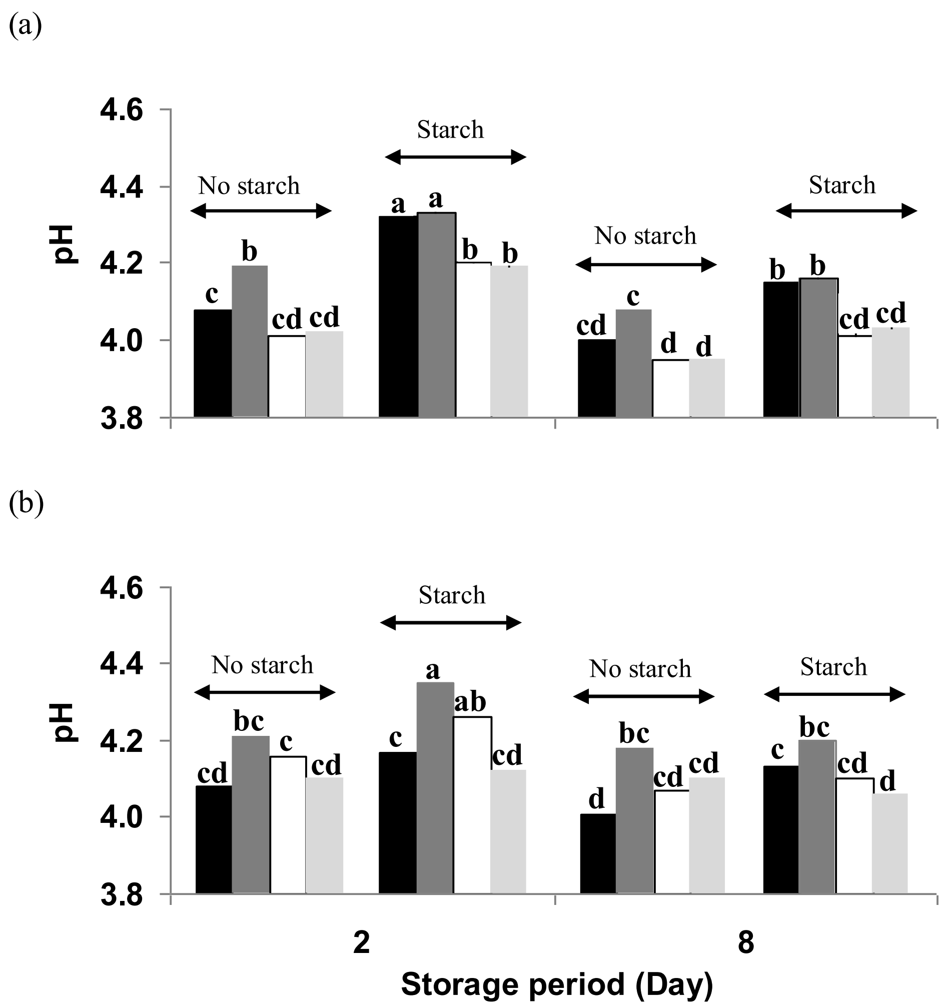
Table 2. Elastic modulus (G′) at 1 Hz of set and stirred yoghurts made with or without starch and fermented with EPS-producing lactic acid bacteria

Starter	**Condition**	**G' at 1 Hz (Pa)**	
		Set	**Stirred**
Control	No starch	304.54^{bc}	30.14^{cd}
	Starch	305.57^{bc}	78.53^{b}
LB1	No starch	277.04^{c}	34.51^{cd}
	Starch	373.16^{c}	67.40^{bc}
LB2	No starch	357.52^{abc}	29.91^{cd}
	Starch	412.09^{a}	97.76^{b}
ST1	No starch	369.26^{ab}	25.18^{d}
	Starch	409.53^{a}	148.43^{a}

Values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Each data is the mean of three experiments.

Figure 1



(a)
4.6
4.4
4.2
4.0
3.8
pH
No starch
Starch
No starch
Starch
c
b
cd
cd
a
a
b
b
cd
c
d
d
b
b
cd
cd
(b)
4.6
4.4
4.2
4.0
3.8
pH
No starch
Starch
No starch
Starch
cd
bc
c
cd
c
a
ab
cd
d
bc
cd
cd
c
bc
cd
d
2
8
Storage period (Day)

Figure 2

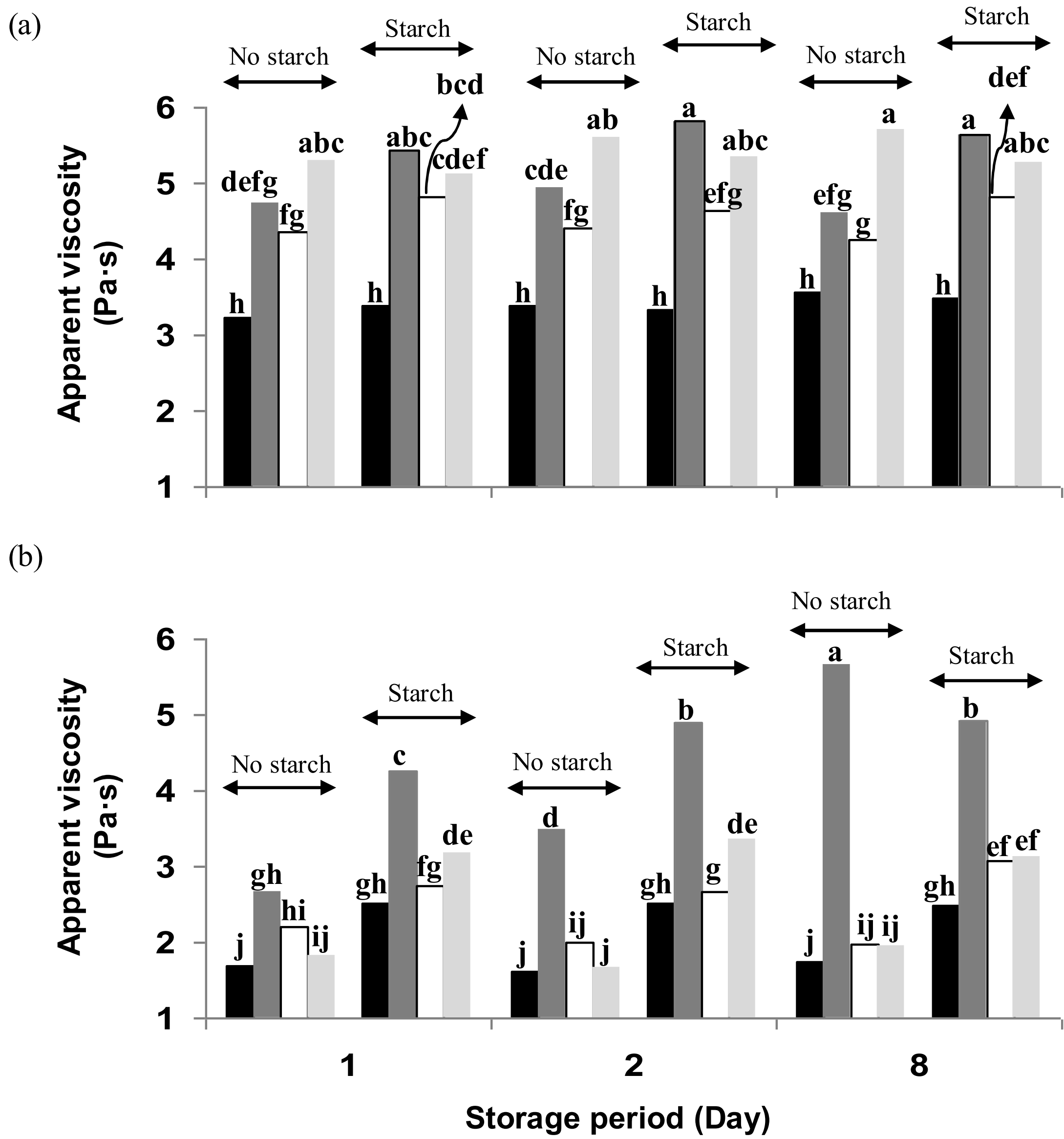
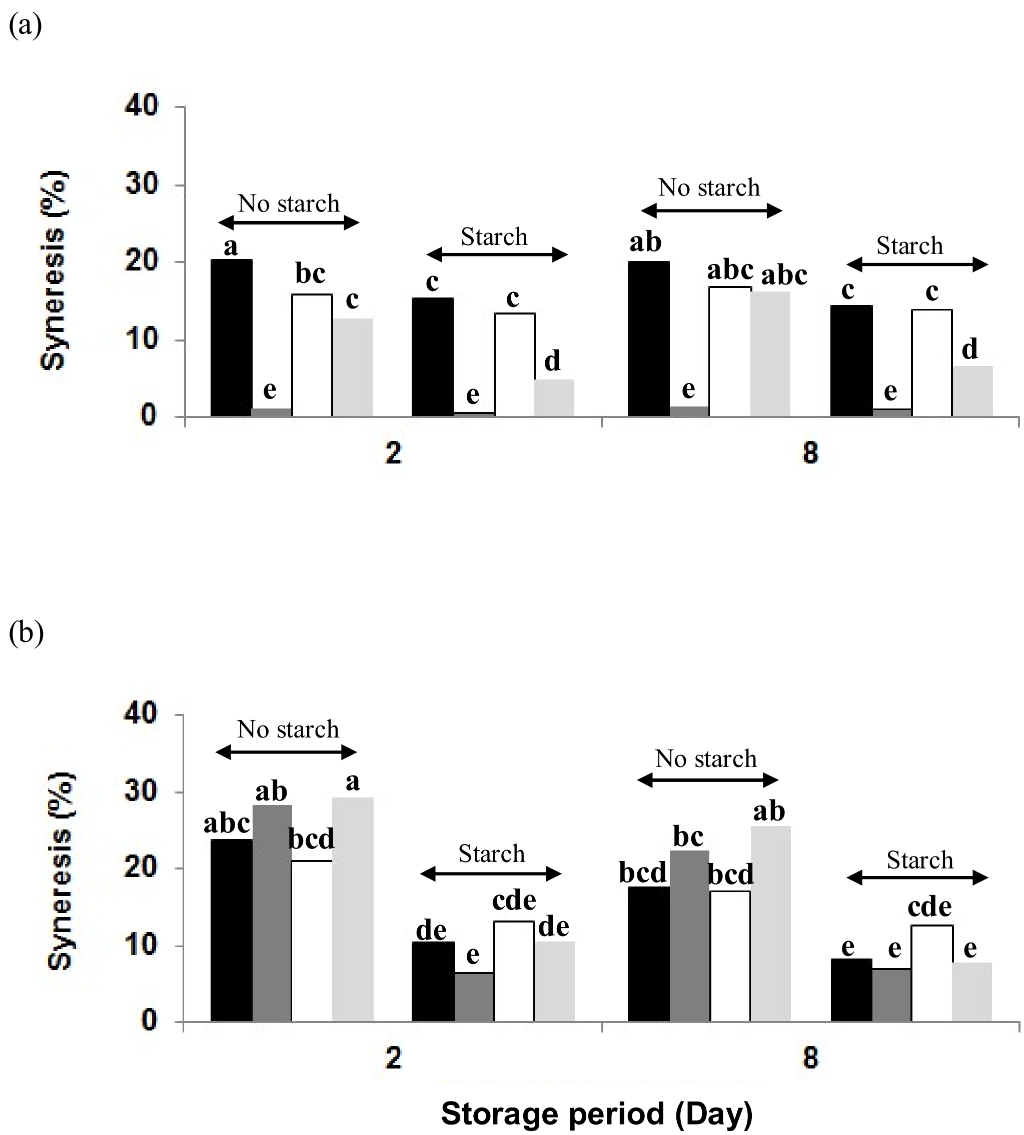
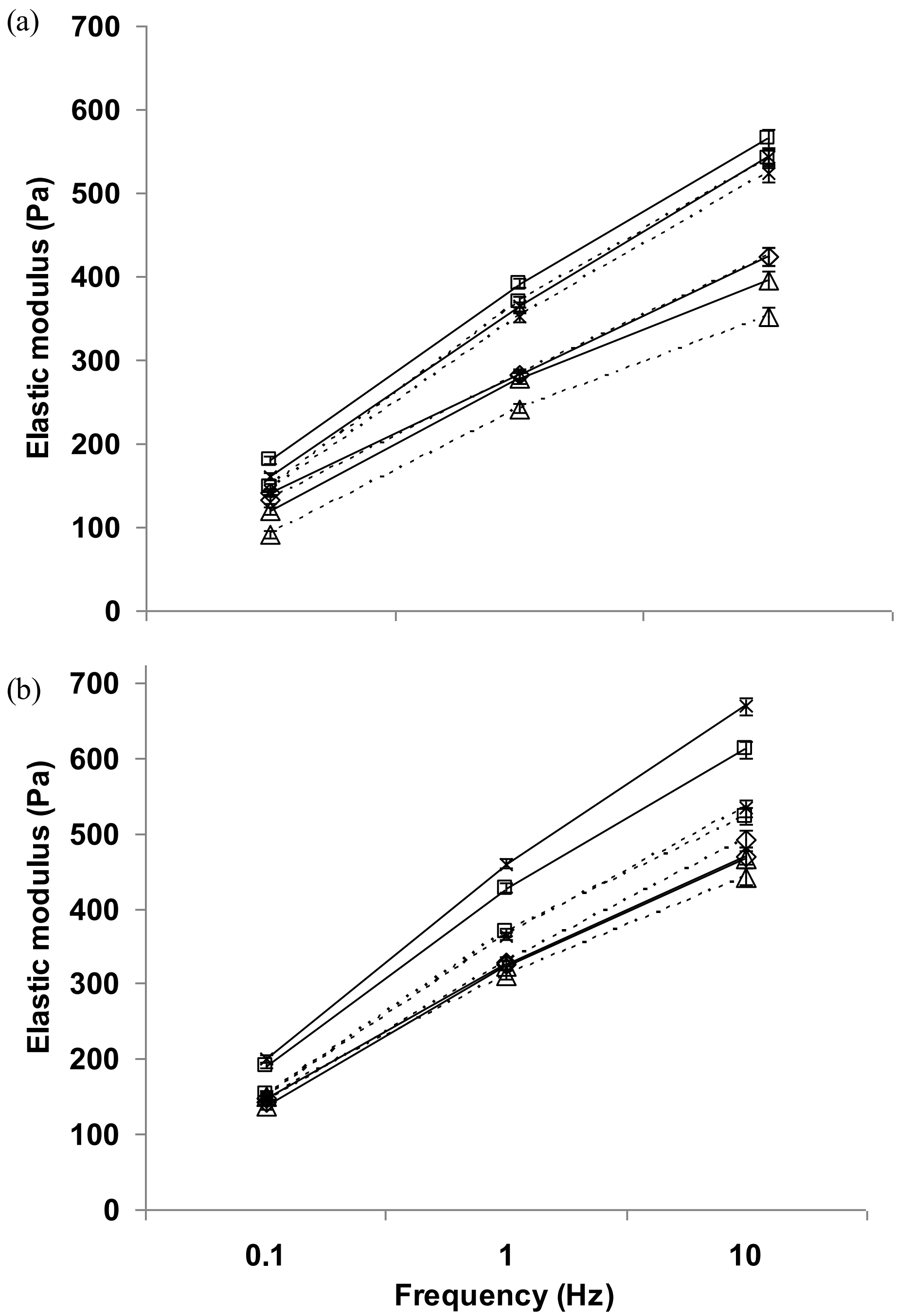

(a)
No starch
Starch
No starch
Starch
No starch
Starch
bcd
def
h
defg
fg
abc
h
abc
cdef
h
cde
fg
ab
h
a
efg
abc
h
efg
g
a
h
a
abc
Apparent viscosity (Pa·s)
6
5
4
3
2
1
(b)
No starch
Starch
No starch
Starch
No starch
Starch
j
gh
hi
ij
gh
c
fg
de
j
d
ij
j
gh
b
g
de
j
a
ij
ij
gh
b
ef
ef
Apparent viscosity (Pa·s)
6
5
4
3
2
1
1
2
8
Storage period (Day)

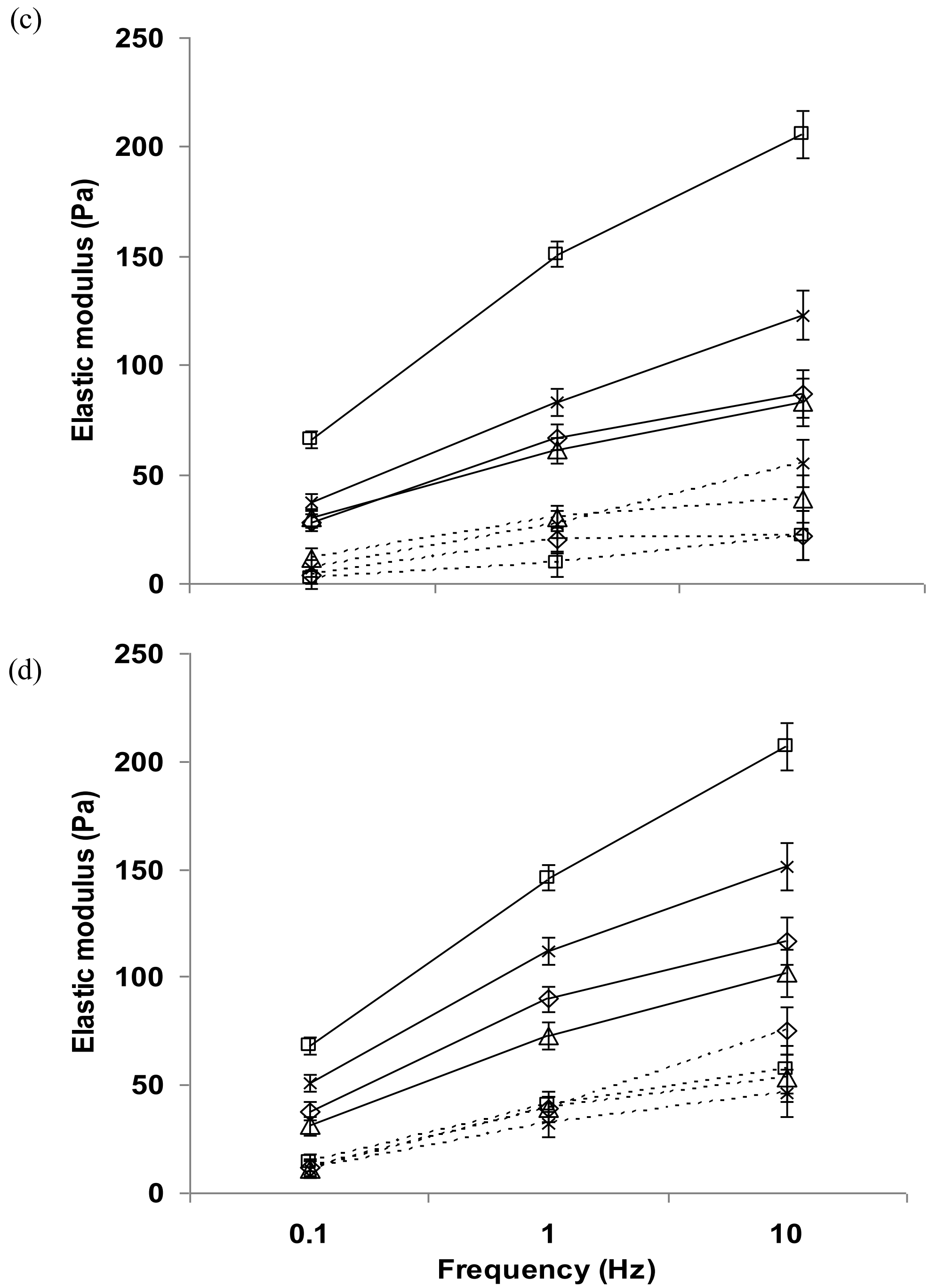
Figure 3



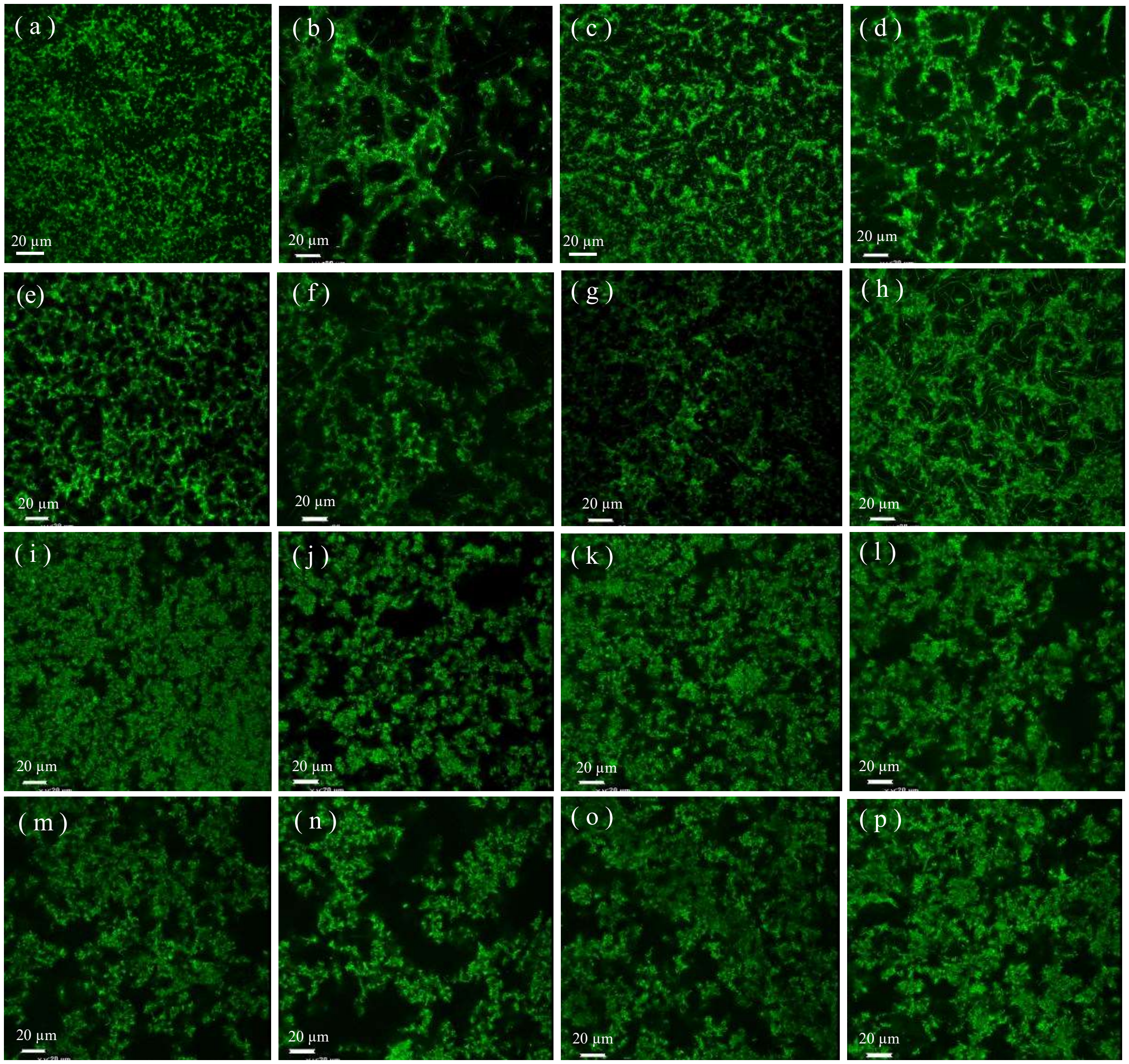
(a)
Syneresis (%)
40
30
20
10
0
No starch
Starch
No starch
Starch
a
e
bc
c
c
e
c
d
ab
e
abc
abc
c
e
c
d
2
8
(b)
Syneresis (%)
40
30
20
10
0
No starch
Starch
No starch
Starch
abc
ab
bcd
a
de
e
cde
de
bcd
bc
bcd
ab
e
e
cde
e
2
8
Storage period (Day)

Supplementary material





Supplementary Fig. S1: Elastic modulus as functions of frequency of set (a-b) and stirred (c-d) yoghurts with 0 (dotted line) and 0.75 (plain line) % modified starch and fermented with starters producing EPS: control (◊), LB1 (Δ), LB2 (×) and ST1 (□) after 2 (a and c) and 8 (b and d) days of storage at 4°C. Each data point is the mean of three experiments. Bars indicate standard error of the mean.



Supplementary Fig. S2: Microstructure of set (a to h) and stirred (i to p) yoghurts made with 0% (a to d and i to l) or 0.75% (e to h and m to p) modified starch and fermented with the control (a, e, i, and m), LB1 (b, f, j, and n), LB2 (c, g, k, and o),or ST1 (d, h, l, and p) starters producing EPS with different structural characteristics.