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Concentration dependent effects of dextran on the physical properties of acid milk gels

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ABSTRACT

The effect of dextran from *Leuconostoc mesenteroides* (DEX_{500}), added to milk prior to acidification with glucono-δ-lactone (GDL) or *Streptococcus thermophilus* DSM20259, was studied with respect to polysaccharide concentration. The incorporation of 5–30 g/kg DEX_{500} significantly affected gelation behavior. Increasing DEX_{500} concentrations resulted in a linear increase of gel stiffness (GDL gels: $R^2 = 0.96$; microbial acidification: $R^2 = 0.94$; $P < 0.05$) and 30 g/kg DEX_{500} resulted in a 2-fold higher stiffness compared to gels without polysaccharide. The respective stirred gels depicted a significant reduction in syneresis, which decreased from 30.4% (0 g/kg DEX_{500}) to 22.0% (30 g/kg DEX_{500}) for chemically acidified gels after 1 d of storage. Physical characteristics of DEX_{500} in aqueous solution were helpful to explain its behavior in the complex system milk.

1. Introduction

Texture is an important quality attribute of yogurt and yogurt-like products made from milk by fermentation with lactic acid bacteria (LAB), which can be modified by adding stabilizers or by taking advantage of *in situ* produced exopolysaccharides (EPS) (Cerning, 1995; Tamime & Robinson, 1999). Texturizers for fermented dairy products comprise charged (*e.g.*, xanthan, carrageenan, pectin) or uncharged polysaccharides (*e.g.*, guar or locus bean gum, β-glucan) of different origin which are expected to contribute to a smooth texture, to enhance viscosity and to reduce whey separation. In many experimental studies, however, such additives led to softer, more unstable products. For example, Sanchez, Zuniga-Lopez, Schmitt, Despond and Hardy (2000) showed that the addition of 1 g/L locus bean gum altered gel microstructure toward a more porous network, whereas the addition of 5 g/L guar gum or locus bean gum increased apparent viscosity, but lowered gel stiffness (Everett & McLeod, 2005).

EPS produced by dairy LAB are usually uncharged (Vaningelgem et al., 2004), and only low amounts (13–170 mg/L) are necessary to affect product texture. Besides enhanced viscosity and creaminess these polysaccharides also reduce syneresis because of their water binding ability (Folkenberg, Dejmek, Skriver, & Ipsen, 2005; Marshall & Rawson, 1999). Other factors that come into play is ropiness, and whether the EPS are associated with the protein network or located in pores (Folkenberg et al., 2005; Folkenberg, Dejmek, Skriver, & Ipsen, 2006; Hassan, Ipsen, Janzen, & Qvist, 2003). The effects of *in situ* produced EPS have mainly been related to their structure (Folkenberg et al., 2006; Gentes, St-Gelais, & Turgeon, 2011; Ruas-Madiedo, Hugenholtz, & Zoon, 2007). However, we showed in a previous study that the effects of the addition of purified neutral EPS from *S. thermophilus* ST-143 to milk prior to acidification on gel stiffness and viscosity are concentration dependent, and that even amounts of 150 mg/L EPS are sufficient to improve rheological properties (Mende, Peter, Bartels, Rohm, & Jaros, 2013). The addition of charged EPS from marine bacteria influenced gel properties as well, whereas no concentration effect was observed (Girard & Schaffer-Lequart, 2007a).

The stability of the microstructure of acid milk gels depends on the physico-chemical properties of the proteins and polysaccharides (molecular mass, charge, molecular conformation distinguished by chain length, chain stiffness, branching), their ratio, but also on interactions between the macromolecules (Doublier, Garnier, Renarda, & Sanchez, 2000; de Kruif & Tuinier, 2001). Anionic, adsorbing polysaccharides interact with casein at the surface of the micelles through electrostatic interactions; consequently, pH is an important parameter. In the presence of neutral, non-adsorbing polysaccharides direct interactions with proteins play a minor role, whereas depletion effects may induce phase separation (Tuinier, ten Grotenhuis, & de Kruif, 2000). In that case, pH only affects protein self association (Doublier et al., 2000; Syrbe, Bauer, & Klostermeyer, 1998), and the stability of the protein network is based on the competition between phase separation and

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protein gelation (Tavares, Monteiro, Moreno, & Lopes de Silva, 2005).

We are particularly interested in the understanding of interactions between uncharged, non-gelling polysaccharides and proteins in milk gels. Commercially available are only EPS from non-dairy LAB (*e.g.* dextran, levan, alternan or reuteran) and among them dextran is the only one approved for use in the food industry (European Commission, 2001). Dextran, mainly utilized in pharmaceutics, medicine, chemical analytics, cosmetics, coatings or paints, is polydisperse with a molecular mass varying from 10^5 to 10^9 Da; defined molecular mass may be obtained by acid or enzymatic hydrolysis (Bovey, 1959; Naessens, Cerdobbel, Soetaert, & Vandammeet, 2005; Rehm, 2010). Commercial dextran is mainly class I from *Leuconostoc mesenteroides* B-512F with a backbone of α-(1,6)-linked glycosyl residues, and approximately 5% α-(1,3)-linked side chains (50–100 residues) (Jeanes et al., 1954; Naessens et al., 2005). It is a flexible, random coil polysaccharide which entangles and aggregates when solution concentration increases (Ioan, Aberle, & Burchard, 2001; McCurdy, Goff, Stanley, & Stone, 1994; Pinder, Swamson, Hebraud, & Hemar, 2006). In aqueous solution, dextran generally exhibits Newtonian behavior up to rather high concentration; ionic strength and pH do not affect viscosity (Kasaai, 2012; McCurdy et al., 1994; Tirtaatmadja, Dunstan, & Boger, 2001).

The application of dextran in milk products is hardly investigated. To the best of our knowledge, only Girard and Schaffer-Lequart (2007a) applied dextran, but in a low concentration (0.5 g/L), where no effects were observed. Encouraged from preliminary experiments the aim of this study was to investigate the effects of different concentrations of dextran on the gelation behavior and on the rheological properties of acidified milk gels and link them to the physical characteristics of the polysaccharide in aqueous solution.

2. Materials and methods

2.1. Materials

Reconstituted skim milk was prepared from low-heat skim milk powder (SMP; Alpavit Käserei Champignon Hofmeister GmbH & Co. KG., Lauben/Allgäu, Germany). Glucono-δ-lactone (GDL) and dextran, molecular mass: 5×10^5 Da (DEX_{500}), was obtained from Sigma–Aldrich GmbH (Taufkirchen, Germany). Microbial acidification was performed with EPS negative *Streptococcus thermophilus* DSM20259 (Mårtensson, Öste, & Holst, 2002) from Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). Milk serum was obtained by two-step ultrafiltration of reconstituted skim milk (120 g/kg dry matter) using a 0.1 μm polyethersulfone membrane and a 10 kDa Hydrosart membrane (Sartorius AG, Göttingen, Germany).

2.2. Characterization of aqueous dextran solutions

1, 5, 10, 15, 20 or 25 g/L DEX_{500} were dissolved in deionized water, 0.1 or 1.0 mol/L NaCl, or milk serum, and filled into the 1.59 mm capillary of a LOVIS rolling ball microviscometer (Anton Paar GmbH, Ostfildern, Germany). After equilibration to 20 °C, rolling time t was measured at an angle of 70° in two independently prepared solutions using gold-coated steel balls (d = 1.5 mm, ρ = 7.88 g/mL) in six-fold each. Specific viscosity η_{sp} (−) as the solute's contribution to viscosity was calculated by

$$\eta_{sp} = \frac{t_{Sample}}{t_{Solvent}} - 1 \quad (1)$$

Intrinsic viscosity $[\eta]$ (mL/mg) was estimated on the basis of the truncated Huggins (Eq. (2)) or Kramer (Eq. ((3)) equations (Sudduth, 1998) and their respective constants K_H and K_K by plotting η_{sp}/c or $[\ln(\eta_{sp} + 1)]/c$ *versus* DEX_{500} concentration c_{DEX} (g/L), and extrapolating to zero polymer concentration:

$$\frac{\eta_{sp}}{c} = [\eta] + K_H \cdot [\eta]^2 \cdot c \quad (2)$$

$$\ln \frac{\eta_{sp} + 1}{c} = [\eta] + K_K \cdot [\eta]^2 \cdot c \quad (3)$$

Shear viscosity measurements were performed for DEX_{500} in deionized water (5–300 g/L) at 20 °C using an AR-G2 rheometer (TA Instruments, Eschborn, Germany) with a concentric cylinder device (d_i = 28 mm, d_a = 30 mm, h = 42 mm) and Peltier temperature control. Shear rate $\dot{\gamma}$ was increased from 0.1 s^{-1} to 100 s^{-1} in 10 steps per decade. At each data point, pre-shearing was 20 s, and measurement time was 5 s. Critical overlap concentrations of dextran were determined from plots of log specific viscosity in shear ($\eta_{sp,S} = \eta_{DEX}/\eta_{Water} - 1$) *vs.* log ($c\,[\eta]$) (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981).

2.3. Preparation of acid milk gels

800 g reconstituted skim milk (150 g/kg dry matter) was transferred into a stainless steel vessel (d = 120 mm, h = 140 mm), heated to 90 °C for 15 min and cooled in ice water. Before acidification, 200 g deionized water (reference) or DEX_{500} solution was added to achieve a final milk solids content of 120 g/kg and a target c_{DEX} of 0, 5, 12.5 or 30 g/kg.

Acidification was induced either by adding 30 g/L GDL to milk of 30 °C which was made as described above, or by adding 30 g/L *S. thermophilus* DSM20259 starter culture to milk which was adjusted to 37 °C. The starter was prepared anaerobically at 40 °C in MRS broth (de Man, Rogosa & Sharpe, 1960; Merck KGaA, Darmstadt, Germany), followed by a second incubation in enriched skim milk (10 g/L SMP, 10 g/L casein peptone, 5 g/L yeast nitrogen base). At the end of acidification (3 h for GDL, pH 4.6 for *S. thermophilus*), the vessel was transferred into ice water for 10 min. Subsequently, the gel was broken by applying a defined stirring protocol (Mende, Mentner, Thomas, Rohm, & Jaros, 2012), poured into beakers and stored at 6 °C.

2.4. Rheology of set and stirred milk gels

Rheological characterization of microbially acidified gels was carried out with an ARES RFS3 rheometer (TA Instruments, Eschborn, Germany), and of GDL gels with the AR-G2. The RFS3 was equipped with a concentric cylinder device (d_i = 32 mm, d_a = 34 mm, h = 33.5 mm) and a water bath for temperature control (geometry for the AR-G2, see above). Gelation was monitored in dynamic mode by measuring storage modulus G' (Pa) as a function of time. Strain amplitude was set to 0.003, and angular frequency was 1 rad/s. Subsequently, a strain sweep at 1 rad/s was carried out. Stirred gels were characterized by thixotropic loop experiments where the shear rate was linearly increased from 0 to 100 s^{-1} within 100 s, and subsequently reduced to zero within another 100 s. For flow curve measurements, shear rate was increased from 0.03 s^{-1} to 100 s^{-1} with 5 points per decade; after 140 s pre-shearing, viscosity readings were recorded during 5 s.

2.5. Permeability and forced syneresis

The permeability of GDL gels was measured by the tube method (Jaros, Rohm, Haque, Bonaparte, & Kneifel, 2002; van Marle & Zoon, 1995). Gels of a height of approximately 50 mm were formed in glass tubes (d = 4 mm, h = 250 mm) by acidification with GDL (30 °C for 3 h) and stored at 6 °C overnight. The tubes were then transferred into a perspex bath with milk serum (T = 20 °C, pH 4.0) and

Table 1
Intrinsic viscosity $[\eta]$, hydrodynamic coil radius R_h, and coil overlap concentration $c_{CO} = 2.5/[\eta]$ for DEX_{500} in different solvents at 20 °C.[a]

Solvent	$[\eta]$ (mL/mg)	R_h (nm)	c_{CO} (mg/mL)
Huggins equation: $\eta_{sp}/c = [\eta] + k_H[\eta]^2$			
Deionized water	0.039[a]	14.6[a]	64.0[a]
0.1 mol/L NaCl	0.040[b]	14.7[b]	62.4[b]
1.0 mol/L NaCl	0.042[c]	14.9[c]	60.2[c]
Milk serum	0.045[d]	15.4[d]	55.2[d]
Kramer equation: $\ln(\eta_{rel})/c = [\eta] - k_K[\eta]^2$			
Deionized water	0.040[a]	14.7[a]	62.0[a]
0.1 mol/L NaCl	0.041[b]	14.8[b]	61.1[b]
1.0 mol/L NaCl	0.043[c]	15.0[c]	58.3[c]
Milk serum	0.046[d]	15.4[d]	54.4[d]

[a] Mean values with different letters in a column block differ significantly ($P < 0.05$).

the fluid level was adjusted to being above the top of the gels to induce a pressure gradient. After defined times the height of the serum column that was present above the gels was measured using a traveling microscope and an MT25 extensiometer (5 μm accuracy; Heidenhain GmbH, Traunreut, Germany). For that purpose, the extensiometer was attached to the optical system that was mounted on vertical rails and moved through screws. The permeability coefficient B (m^2) was estimated graphically for each tube:

$$B = -\ln\frac{h_E - h_{t(x)}}{h_E - h_{t(x-1)}} \cdot \nu \cdot h_G \cdot \frac{1}{g \cdot (t_x - t_{x-1})} \quad (4)$$

In Eq. (4), h_E (m) is the height of milk serum in the empty reference tube, and h_G (m) is the height of the initial gel. The level of serum passing through the gel, measured at time t_x or t_{x-1} (s), was $h_{t(x)}$ and $h_{t(x-1)}$ (m), respectively, g refers to acceleration of gravity, and ν (m^2/s) is the kinematic viscosity of the milk serum. All experiments were performed in triplicate with 12 tubes in each run. Outliers were identified by Dixon tests and excluded from further calculations.

Syneresis of set and stirred acidified milk was determined in triplicate using a centrifugation method (6 °C, 1000 × g, 20 min; Jacob, Nöbel, Jaros, & Rohm, 2011). After centrifugation (set gels were prepared in centrifuge tubes), the expelled whey was removed with a pasteur pipette. Syneresis S (%) is expressed as the fraction of separated whey.

2.6. Statistical analysis

SYSTAT 12 (Systat Software GmbH, Erkrath, Germany) was used for performing ANOVA and difference testing. Unless stated otherwise, data are arithmetic mean ± half deviation range ($n = 2$) or arithmetic mean ± standard deviation ($n > 2$). Significance statements refer to an error probability of $P < 0.05$.

3. Results and discussion

3.1. Intrinsic viscosity and critical overlap concentration of dextran

Intrinsic viscosity of DEX_{500} in water calculated by Eqs. (2) and (3) was 0.039 and 0.040 mL/mg, respectively. Generally, $[\eta]$ obtained by the Kramer equation was slightly higher (Table 1). In the presence of salts, averaged $[\eta]$ increased to 0.042 mL/mg (1.0 mol/L NaCl) and 0.046 mL/mg (milk serum). $[\eta]$ in 0.1 mol/L NaCl, showing a ionic strength close to that of milk (Daviau, Famelart, Pierre, Goudedranche, & Maubois, 2000), was 0.041 mL/mg. Although being significant, these differences can be regarded as small, especially when considering the low absolute values in comparison to other polysaccharides (*e.g.* dextrose, amylose, xanthan, guar gum) with $[\eta]$ between 0.1 and

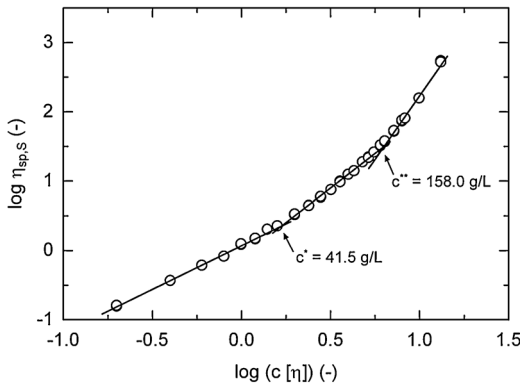

Fig. 1. Specific viscosity $\eta_{sp,S}$ from shear experiments ($n = 2$) of DEX_{500} in deionized water (20 °C) as affected by concentration c. Critical overlap concentrations c^* and c^{**} separate the graph into three regions: (1) $c < c^*$, $\eta_{sp,S} = 1.25\,(c\,[\eta]) + 0.08$ ($R^2 = 0.997$); (2) $c^* < c < c^{**}$, $\eta_{sp,S} = 2.00\,(c\,[\eta]) - 0.09$ ($R^2 = 0.994$); (3) $c > c^{**}$, $\eta_{sp,S} = 3.68\,(c\,[\eta]) - 1.43$ ($R^2 = 0.991$).

5 mL/mg (Arvidson, Rinehart, & Gadala-Maria, 2006). $[\eta]$ of dextran with comparable molecular mass is given with 0.04–0.08 mL/mg (Antoniou & Tsianou, 2012; Kasaai, 2012; McCurdy et al., 1994; Tirtaatmadja et al., 2001). Such a variation may be attributed to differences in manufacture, which significantly influence glycosidic bonds and branching and, consequently, the polymer's hydrodynamic behavior (Lefebvre & Doublier, 2005).

The intrinsic viscosity represents the volume that is occupied by a macromolecule in a highly diluted system and depends on the molecular structure (linear or branched, rigid or flexible) and the molecular mass of a particular polysaccharide (Kulicke & Clasen, 2010). A low $[\eta]$ accompanied with a high molecular mass implies a small occupied volume and, consequently, a more compact shape of the polysaccharide in solution. The hydrodynamic coil radius R_h, estimated from $[\eta]$ by (Antoniou, Themistou, Sarkar, Tsianou, & Alexandridis, 2010; Antoniou & Tsianou, 2012)

$$R_h = \sqrt[3]{\frac{3 \cdot [\eta] \cdot m^m}{10 \cdot \pi \cdot N_{AV}}} \quad (5)$$

where m^m is molar mass and N_{AV} is Avogadro number, was approximately 15 nm (see Table 1). Consequently, the volume occupied by a coil is 13.0×10^3–15.3×10^3 nm^3. The coil overlap concentration – the particular concentration where polysaccharide molecules start to interact – was calculated by $c_{CO} = 2.5/[\eta]$ (Antoniou et al., 2010); c_{CO} was ~64 mg/mL in deionized water, decreased with increasing ionic strength, and was ~55 mg/mL in milk serum as solvent.

The specific behavior of a polysaccharide can be illustrated by plotting η_{sp} *vs.* the product of concentration and $[\eta]$, which can be interpreted as space occupancy (Arvidson et al., 2006; Lefebvre & Doublier, 2005; Morris et al., 1981). In log scale, our experimental data – specific viscosity from shear experiments ($\eta_{sp,S}$) of DEX_{500} in water at different c_{DEX} – showed three regions (Fig. 1), separated by two critical concentrations at space occupancies of 1.6 and 6.3. The corresponding $c^* = 41.5$ mg/mL and $c^{**} = 158.0$ mg/mL, respectively, are comparable to those reported for dextran with similar molecular mass (Lefebvre & Doublier, 2005; McCurdy et al., 1994; Pinder et al., 2006). The slope of $\eta_{sp,S}$ *vs.* $(c\,[\eta])$ was 1.3 in the diluted ($c < c^*$), 2.0 in the semi-diluted ($c^* < c < c^{**}$), and 3.7 in the concentrated domain ($c > c^{**}$), indicating that DEX_{500} in water behaves as random coil polymer (McCurdy et al., 1994; Morris et al., 1981; Pinder et al., 2006). Since $[\eta]$ was only slightly higher in milk serum than in deionized water, it can be expected that DEX_{500} behaves as random coil polymer in milk as well.

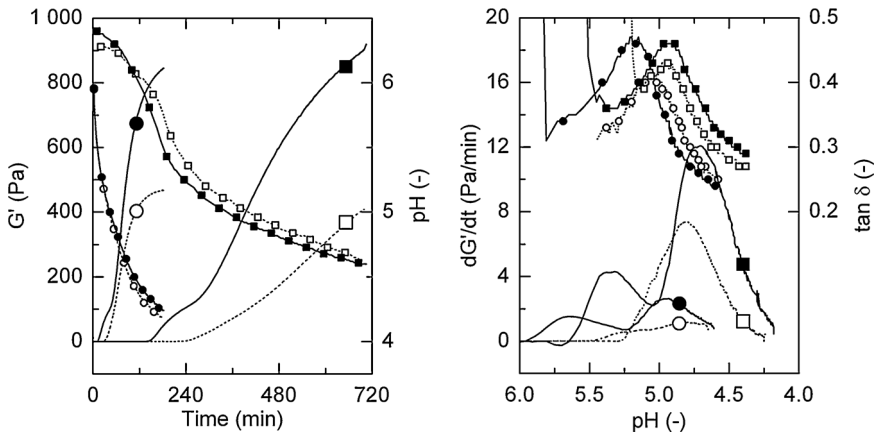

Fig. 2. Development of gel stiffness G' (lines and marker indicators) and pH (lines and markers; left graph), and gelation rate dG'/dt (lines and marker indicators) and tan δ (lines and markers; right graph) during acidification. Acidification of milk ($n = 2$) without (open symbols, dotted lines) or with 30 g/kg DEX_{500} (closed symbols, full lines) was induced by glucono-δ-lactone (circles) or *S. thermophilus* DSM20259 (squares).

3.2. Gelation of milk with dextran

Fermentation with *S. thermophilus* DSM20259 at 37 °C took approximately 12 h until pH 4.6. Acidification with 30 g/L GDL at 30 °C was almost 4-times faster (pH 4.3 was reached in 3 h). Chemically acidified milk is frequently used as yogurt model, although it has to be considered that the resulting gels are not completely comparable with respect to their rheological and physical properties (Gentes et al., 2011; Girard & Schaffer-Lequart, 2007a; Mende et al., 2013; van Marle & Zoon, 1995). For neither acidification regime, the acidification rate was affected by the presence of DEX_{500} (Fig. 2). However, G' as a measure of gel stiffness, which is determined by gel structure (number of bonds, length of strands) and interaction forces between structural elements (Lakemond & van Vliet, 2008b), increased faster during acidification of milk containing DEX_{500}. Gelation onset time t_{gel}, defined as the time where G' exceeds 1 Pa (Jaros, Jacob, Otto, & Rohm, 2010) was significantly reduced in the presence of DEX_{500}, so that pH at gelation onset pH_{gel} increased concomitantly (Table 2). After incorporating 30 g/kg DEX_{500} in the base milk, t_{gel} was by a factor of approximately 2 and 1.5 lower compared to the references of chemically or microbially acidified gels, respectively. An altered gelation onset has also been observed in the presence of other polysaccharides, especially in case of anionic or uncharged EPS (Girard & Schaffer-Lequart, 2007a,b; Mende et al., 2013), or starch (Singh & Byars, 2009).

The shift of pH_{gel} is also evident from the first time derivative of G' (gelation rate) dG'/dt *vs.* pH (see Fig. 2). The maximum of dG'/dt was by a factor of approximately 5 – 6 lower in case of milk that was fermented with microorganisms (see Table 2), and increased significantly with c_{DEX} in both types of gels.

A first local maximum of dG'/dt that was observed with increasing c_{DEX} was also reported for gelation profiles of milk with *in situ* produced EPS (Girard & Schaffer-Lequart, 2007b; Mende et al., 2012), or after the addition of different neutral polysaccharides (Lazaridou, Vaikousi, & Biliaderis, 2008; Mende et al., 2013). An explanation of this phenomenon can be derived from the tan δ, which shows a local maximum during acidification (see Fig. 2). The presence of DEX_{500} caused a generally higher tan δ during gelation at a particular pH. Such a shift of tan δ has been related to affinities for structural rearrangements (*e.g.*, changes in type and strength of bonds inside the protein network) that cause a momentary weakening of the network (Lakemond & van Vliet, 2008a; Lucey & Singh, 1998); this weakening was more pronounced in the presence of polysaccharides (Mende et al., 2013).

It has been reported that dextran remains unaffected by changes in pH during acidification (McCurdy et al., 1994), and that no electrostatic interactions between the neutral polysaccharide and the casein micelles in milk occur (Dickinson & Semenova, 1992; Doublier et al., 2000). However, de Kruif and Tuinier (2001) and Li et al. (2008) showed that phase separation due to depletion interactions was induced, and differences in the osmotic pressure between the solvent surrounding polysaccharide molecules and the proteins may cause attractive interactions between the proteins. This could serve as explanation for a more pronounced aggregation of

Table 2
Effect of DEX_{500} concentration c_{DEX} on pH and time at gelation onset (pH_{gel}, t_{gel}), final gel stiffness G'_{end} and maximal gelation rate dG'/dt_{max} during acidification with glucono-δ-lactone or *S. thermophilus* DSM20259.[a,b]

c_{DEX} (g/kg)	pH_{gel} (–)	t_{gel} (min)	G'_{end}[c] (Pa)	dG'/dt_{max}[d] (Pa/min)
Acidification with glucono-δ-lactone				
0.0	5.17[a] ± 0.05	28.9[a] ± 0.5	457[a] ± 12	7.40[a] ± 0.10
5.0	5.20[a] ± 0.04	25.8[b] ± 0.5	543[b] ± 11	8.56[a,b] ± 0.22
12.5	5.27[b] ± 0.03	22.0[c] ± 0.5	694[c] ± 1	10.19[c] ± 0.33
30.0	5.46[c] ± 0.04	12.9[d] ± 0.5	844[d] ± 34	12.22[d] ± 0.74
Acidification with *S. thermophilus* DSM20259				
0.0	5.44[a] ± 0.03	228[a] ± 8	447[a] ± 47	1.16[a] ± 0.03
5.0	5.42[a] ± 0.01	205[a,b] ± 15	587[b] ± 3	1.73[b] ± 0.07
12.5	5.60[b] ± 0.06	185[a,b] ± 10	747[c] ± 40	2.23[c] ± 0.07
30.0	5.78[c] ± 0.02	150[b] ± 5	919[d] ± 18	2.59[d] ± 0.11

[a] Data are mean ± standard deviation of two independent fermentations.
[b] Means with different letters in a column block differ significantly ($P < 0.05$).
[c] G'_{end} after 3 h acidification with glucono-δ-lactone (pH ~ 4.3), and at pH of 4.6 for acidification with *S. thermophilus* DSM20259.
[d] Maximum of the first derivative dG'/dt.

the casein micelles in the presence of DEX_{500} and an earlier onset of gelation. As indicated by more noticeable structural rearrangements (see changes in tan δ, and in the dG'/dt functions), DEX_{500} appears to interfere with casein aggregation in the early stage of gelation. These structural arrangements lead to a temporary weakening of the milk gels, caused by the competition between phase separation and gelation.

3.3. Physical properties of set milk gels

Final gel stiffness G'_{end} increased almost linearly with DEX_{500} concentration (see Table 2) from 457 Pa (reference) to 844 Pa (c_{DEX} = 30 g/kg) for GDL gels (R^2 = 0.96; P < 0.05), and corresponding stiffness of microbially acidified gels (R^2 = 0.94; P < 0.05) was 447 Pa and 919 Pa, respectively. Similar effects on milk gel stiffness were observed after the addition of charged EPS from marine bacteria (Girard & Schaffer-Lequart, 2007a) or uncharged EPS from *S. thermophilus* (Mende et al., 2013). In contrast, Everett and McLeod (2005) and Lazaridou et al. (2008) showed that several other polysaccharides (guar gum, locus bean gum, β-glucan) weakened the gels because they hindered the proteins to form a continuous network. The DEX_{500} molecules used in our experiments that are small compared to casein micelles did apparently not hinder protein aggregation but rather enhanced it because of depletion interactions, consequently resulting in denser and stiffer protein gels.

The loss tangent at pH 4.6 ($\tan\delta_{pH\ 4.6}$) varied, depending on the acidulant, from 0.30 to 0.33 (chemical acidification) and from 0.26 to 0.24 (fermentation; data not shown). A lower tan δ points on a more solid-like character of a gel and is frequently accompanied with higher stiffness (Kristo, Biliaderis, & Tzanetakis, 2003; Rohm & Kovac, 1994). Chemically induced gels with DEX_{500} exhibited lower G'_{end} than the fermented samples. In line with that the higher $\tan\delta_{pH\ 4.6}$ in chemical gels implies a more viscous character and a coarser network (Lakemond & van Vliet, 2008a), which may be explained by the enhanced acidification rate (Kristo et al., 2003; Lucey, van Vliet, Grolle, Geurts, & Walstra, 1997).

Fig. 3 shows that, in plots of stress amplitude σ *vs.* strain amplitude γ, the chemically acidified reference gels exhibited linearity up to a critical strain $\gamma_C \sim 0.2$, and that DEX_{500} addition shifted γ_C up to approximately 0.5 for c_{DEX} = 30 g/kg. Similar results were obtained for microbially acidified gels. The extended linearity of σ *vs.* γ implies that the gels exhibit a higher structural stability when DEX_{500} is present. The higher energy that is necessary to break casein strands in the presence of polysaccharides indicates that there are more interactions or stronger bonds within the protein network (Lakemond & van Vliet, 2008b).

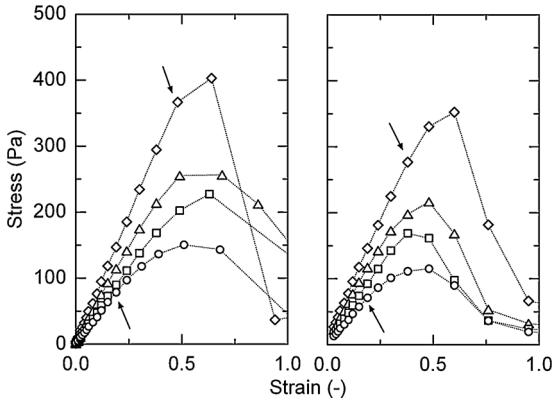

Fig. 3. Shear stress amplitude σ *vs.* shear strain amplitude γ (n = 2) for set milk gels acidified with glucono-δ-lactone (left) and *S. thermophilus* DSM20259 (right). DEX_{500} added prior to acidification: circles, control; squares, 5 g/kg; triangles, 12.5 g/kg; diamonds, 30 g/kg. Arrows indicate end of linearity between σ and γ.

3.4. Characterization of stirred milk gels

Areas A_H between upward and downward flow curves in thixotropic loop experiments significantly increased from 225 and 454 Pa/s (reference) to 663 Pa/s and 1073 Pa/s (30 g/kg DEX_{500}) for chemically and microbially acidified gels, respectively (Table 3). The respective coefficients of determination are R^2 = 0.91 (P < 0.05; chemical acidification) and R^2 = 0.98 (P < 0.05; microbial acidification). The same is true for apparent viscosity η_{app} at $\dot{\gamma} = 100\,s^{-1}$, and a linear correlation between A_H and η_{app} was also evident (R^2 = 0.97; P < 0.05). A higher A_H indicates enhanced shear induced structural breakdown, accompanied by a lower ability to regain initial gel structure (Folkenberg et al., 2006; Jaros, Haque, Kneifel, & Rohm, 2002). Hence, the stronger network formed in milk gels that contained DEX_{500} required a concentration dependent higher energy for breakdown. We assume that shearing leads to the formation of polysaccharide channels and higher product viscosity. Consequently, the recovery of the protein network is hindered (Hassan et al., 2003). Lower A_H and η_{app} of chemically acidified gels point again on differences in the protein network, which are presumably induced by the acidification rate (Renan, Arnoult-Delest, Pâquet, Brulé, & Famelart, 2008).

The flow curves depicted in Fig. 4 are typical for stirred yogurt products (Afonso, Hes, Maia, & Melo, 2003; Jaros, Heidig, & Rohm, 2006) and show a clear shift toward higher shear stress for milk gels with 30 g/kg DEX_{500}. At intermediate DEX_{500} concentration, intermediate stress levels were recorded (data now shown). An almost

Table 3
Hysteresis loop area A_H, apparent viscosity η_{app} and forced whey syneresis S of stirred milk gels with different DEX_{500} concentrations c_{DEX} added to milk prior to acidification with glucono-δ-lactone or *S. thermophilus* DSM20259 after storage at 6 °C for 1 d and 21 d.[a,b]

c_{DEX} (g/kg)	A_H[c] (Pa/s)	A_H[c] (Pa/s)	η_{app}[d] (Pa.s)	η_{app}[d] (Pa.s)	S (%)	S (%)
	1 d	21 d	1 d	21 d	1 d	21 d
Acidification with glucono-δ-lactone						
0.0	225[a] ± 2	568[a] ± 52	239[a] ± 1.5	274[a] ± 0.7	30.4[a] ± 1.4	32.6[a] ± 0.4
5.0	371[b] ± 5	526[a] ± 10	285[b] ± 1.7	336[b] ± 1.0	31.3[a] ± 2.5	24.8[b] ± 0.3
12.5	532[c] ± 15	616[a] ± 2	374[c] ± 2.8	419[c] ± 2.1	31.5[a] ± 0.9	20.6[c] ± 0.8
30.0	663[d] ± 32	927[b] ± 1	525[d] ± 2.6	634[d] ± 2.4	22.0[b] ± 0.9	13.8[d] ± 0.6
Acidification with *S. thermophilus* DSM20259						
0.0	454[a] ± 34	686[a] ± 72	348[a] ± 11.7	414[a] ± 17.3	41.2[a] ± 1.7	39.4[a] ± 0.9
5.0	586[b] ± 56	829[b] ± 59	419[b] ± 25.3	493[b] ± 32.8	40.5[a] ± 0.7	36.6[b] ± 1.7
12.5	753[c] ± 30	980[c] ± 17	526[c] ± 23.3	572[c] ± 18.5	36.6[b] ± 0.8	31.7[c] ± 0.8
30.0	1073[d] ± 95	1527[d] ± 96	721[d] ± 62.5	865[d] ± 31.3	26.6[c] ± 1.7	24.1[d] ± 1.5

[a] Data are mean ± standard deviation of two independent fermentations.
[b] Means with different letters in a column block differ significantly (P < 0.05).
[c] Area between upward and downward flow curve obtained from thixotropic loop experiments.
[d] From thixotropic loop experiments (shear rate: 100 s^{-1}).

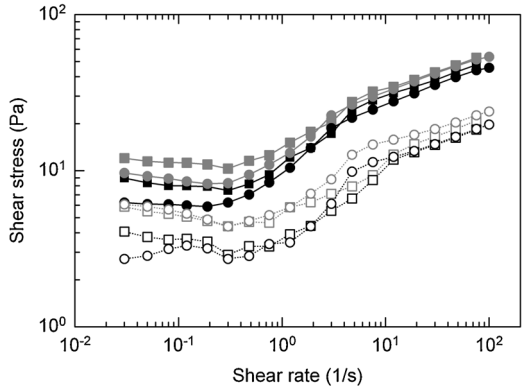

Fig. 4. Flow curves of stirred milk gels acidified with glucono-δ-lactone (circles) and *S. thermophilus* DSM20259 (squares). Acidification of milk ($n = 2$) without (open symbols, dotted lines) or with 30 g/kg DEX_{500} (closed symbols, full lines) added to milk prior to acidification. Product storage was 1 d (black) and 21 d (gray) at 6 °C.

similar behavior was observed for chemically and microbially acidified gels, especially at high shear rates. At low $\dot{\gamma}$, stress was nearly constant, pointing on the existence of a yield stress τ_0 (Jaros et al., 2006). The change in the slope of the flow curves at $\dot{\gamma} \sim 10\,s^{-1}$, that can be interpreted as the domain where large casein aggregates disrupt into smaller ones (Afonso et al., 2003), is less pronounced in gels containing DEX_{500}.

We modeled the flow curves by fitting the region of $0.03\,s^{-1} < \dot{\gamma} < 10\,s^{-1}$ to Herschel Bulkley, and $10\,s^{-1} < \dot{\gamma} < 100\,s^{-1}$ to power law. τ_0 increased from ~3.0 Pa (reference) to 5.1 Pa (chemically acidified) and up to 7.8 Pa (microbially acidified) for gels with 30 g/kg DEX_{500} (1 d storage). The power law exponent n was independent of c_{DEX}, whereas the consistency index K doubled after addition of 30 g/kg DEX_{500}. Furthermore, storage of the milk gels for 21 d enhanced τ_0 and K, which points on a partial structure stabilization of the gel network. Higher A_H and η_{app} after storage confirm this observation. We found a similar flow behavior after addition of purified neutral EPS from *S. thermophilus* (Mende et al., 2013).

3.5. Syneresis and permeability

Permeability B of chemically acidified milk gels decreased linearly ($R^2 = 0.92$; $P < 0.05$) with increasing c_{DEX} from $5.89 \times 10^{-14}\,m^2$ (reference) to $4.21 \times 10^{-14}\,m^2$ (30.0 g/kg DEX_{500}), whereas η_{app} of the respective base milks increased from 3 to 10 mPa s (Fig. 5). Gel permeability provides information on gel structure homogeneity,

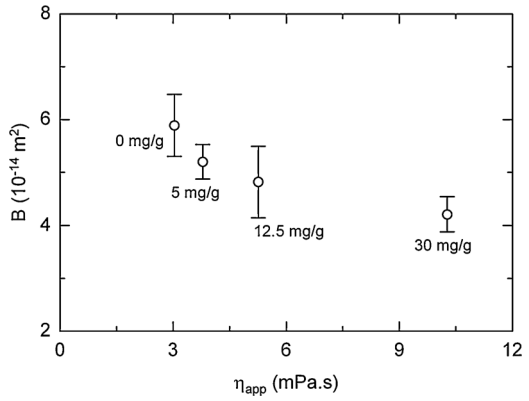

Fig. 5. Permeability coefficient B ($n = 3$) of set milk gels after acidification with glucono-δ-lactone as a function of apparent milk viscosity η_{app} with different concentration of DEX_{500} added prior to acidification. c_{DEX} is denoted at each point.

which depends on the number and size of particles and pores. A lower permeability was often associated with a finer meshed protein network and higher water retention (Lucey & Singh, 1998). Ruas-Madiedo and Zoon (2003) detected lower B in gels with *in situ* produced EPS and suspected that this observation mainly originates from a higher serum viscosity rather than from a more homogeneous protein network. The lower B of samples with DEX_{500}, however, did not only result from higher serum viscosity but also from a more dense and consistent protein network as is evident from the higher G'_{end} in the respective set gels.

Forced syneresis S after 1 d was approximately 5% in chemically acidified set gels and hardly changed with increasing c_{DEX}, whereas a significant decrease of S in microbially acidified gels from 14.5% (reference) to 2.8% (30.0 g/kg DEX_{500}) was observed (data not shown). On the other hand, syneresis of stirred milk gels generally decreased with increasing c_{DEX} (1 d storage) (see Table 3). After 21 d, S was significantly lower in gels with DEX_{500}. Several authors reported on a decrease in whey separation after adding stabilizers (starch, inulin, β-glucan) and explained that with a higher viscosity because of the hydrocolloids water binding capacity (Brennan & Tudorica, 2008; Everett & McLeod, 2005; Lazaridou et al., 2008). A further explanation may be the stronger protein network in gels with polysaccharides, which reacts less fragile when applying an external force.

3.6. Suitability of dextran as a model polysaccharide for EPS from LAB

The concentration dependent effects of dextran on rheological and physical properties of acidified milk gels are similar to the effects of an isolated purified EPS from *S. thermophilus* ST-143 (Mende et al., 2013) raising the question whether the homopolysaccharide dextran might be useful as a model EPS even for the heteropolysaccharide types of EPS from LAB. There are no other reports of uncharged and non-gelling polysaccharides showing such behavior in milk gels.

Both DEX_{500} and $EPS_{ST\text{-}143}$ induced an earlier gelation onset compared to the reference without polysaccharide and gelation profiles (changes in tan δ, and gelation rate) were modified in a similar manner, but on a different concentration level. 0.15–0.35 g/kg of $EPS_{ST\text{-}143}$ were necessary to provoke quantitatively similar effects as obtained after adding 5.0–30.0 g/kg of DEX_{500}. In stirred gels both polysaccharides induced an increase of hysteresis loop area and apparent viscosity. The impact of DEX_{500}, on stirred gels was, however, more pronounced.

Both $EPS_{ST\text{-}143}$ and DEX_{500} are neutral polysaccharides that cause phase separation in milk because of depletion effects which enhance the aggregation of casein micelles during gelation (de Kruif & Tuinier, 2001; Doublier et al., 2000; Li et al., 2008). Differences in the polysaccharide's concentration to obtain similar quantitative effects may arise from differences in polysaccharide's structure: molecular mass and intrinsic viscosity of $EPS_{ST\text{-}143}$ in deionized water was 2.6×10^6 Da and 1.14 mL/mg, respectively (Mende et al., 2013), and significantly higher than those of DEX_{500} (0.5×10^6 Da; 0.04 mL/mg). DEX_{500} is a relatively flexible polymer with a small hydrodynamic coil radius (15 nm) and a more compact structure and a lower molecule volume in solution than $EPS_{ST\text{-}143}$ with an approximately 5-fold higher R_h (~80 nm) and less flexible chains. This explains that higher concentrations of DEX_{500} are necessary to affect the aggregation of casein micelles that have an average diameter of approximately 200 nm (Dalgleish, 2011; Horne, 2002). Differences in stirred products may be also explained with the smaller size of DEX_{500} molecules, which do not hinder the rebuilding of the casein network after shearing to the same extent as the larger and stiffer $EPS_{ST\text{-}143}$ molecules.

4. Conclusions

The addition of dextran from *L. mesenteroides* (DEX_{500}) to milk prior to acidification with glucono-δ-lactone or a *S. thermophilus* strain revealed distinct concentration dependent effects on the rheological and physical properties of the gels. In set gels increasing DEX_{500} concentrations led to stiffer gels with a more dense and homogeneous structure, indicated by an increasing storage modulus and decreasing permeability. Stirred systems with DEX_{500} showed reduced syneresis and increased viscosity and were more stable against shearing. Physical parameters of the polymer in aqueous solution (coil overlap concentration, intrinsic viscosity) are helpful to explain its behavior in the more complex system milk. Further experiments including imaging should be carried out to gain more information on similarities and differences of DEX_{500} and heteropolysaccharides of LAB.

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