

Addition of Exopolysaccharides from *S. Thermophilus* or Dextran to Milk prior to Acidification: A Comparative Study

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ABSTRACT

The addition of the neutral polysaccharides dextran₅₀₀ (*Leuconostoc mesenteroides*) and EPS_{ST-143} (*Streptococcus thermophilus*) significantly affected gelation behaviour and rheological properties of microbially acidified milk gels. However, a much lower concentration of EPS_{ST-143} was necessary to achieve similar gel stiffness and product viscosity. This fact was related to the different behaviour of the polysaccharides in solution.

INTRODUCTION

Extracellular bacterial polysaccharides (EPS) are commonly used in the dairy industry to influence textural (viscosity, gel stiffness, syneresis) and sensory properties of the products. They can be used as additives or are produced *in situ* by lactic acid bacteria.¹ The impact of these hydrocolloids mainly depends on their structure (molecular mass, charge, branching, chain flexibility), their concentration, and their interaction with other product components.^{2,3} However, their effect on the rheological properties of milk gels is still not completely understood.

The aim of our study was therefore to compare the rheological properties of aqueous solutions of a purified EPS from *Streptococcus thermophilus* and a commercially available well-defined dextran from *Leuconostoc mesenteroides*. The results should be related to the rheology of

acid milk gels spiked with these polysaccharides.

MATERIALS AND METHODS

Polysaccharides

Dextran from *Leuconostoc* ssp. with a molecular mass of 500 kDa (dextran₅₀₀) was purchased from Sigma-Aldrich (Seelzen, Germany).

EPS used in this study was produced by *S. thermophilus* ST-143 (EPS_{ST-143}; purity ~ 85 %). Isolation and purification was performed as described in Mende et al., 2013.³

Characterisation of the polysaccharides and their aqueous solutions

Molecular mass (M_m) and monosaccharide composition of EPS_{ST-143} were determined by size-exclusion chromatography³ and gas chromatography, respectively. Ion exchange chromatography was used to determine the charge.⁴

Solutions of dextran₅₀₀ ($c = 0 - 330$ g/L) and of EPS_{ST-143} ($c = 0 - 7.7$ g/L) were prepared with deionized water.

Flow curves were measured at 20 °C with an ARG2 rheometer (TA Instruments, Eschborn, Germany) and a concentric cylinder device. The shear rate was increased from 0.1 s⁻¹ to 100 s⁻¹ with 10 points/decade (pre-shearing of 20 s at each data point).

Intrinsic viscosity was determined with a LOVIS rolling ball microviscometer (Anton Paar GmbH, Germany) at 20 °C.

Preparation and characterisation of acid milk gels

The polysaccharides were added to reconstituted skim milk (dry matter 120 g/kg) prior to fermentation with the non EPS producing *Streptococcus thermophilus* DSM20259 (Deutsche Sammlung für Mikroorganismen und Zellkulturen, Germany) at 37 °C.³

Gelation was monitored with an ARES RFS3 rheometer (TA Instruments, Eschborn, Germany) and a concentric cylinder device at $\gamma = 0.003$ and $\omega = 1$ rad/s.

Stirred gels were produced according to a defined protocol^{3,4} and, after storage at 6 °C for 1 d, flow curves were measured.³

Syneresis of set and stirred gels was measured with a centrifugation method.³

RESULTS AND DISCUSSION

Aqueous polysaccharide solutions

Dextran₅₀₀ and EPS_{ST-143} are both water soluble neutral polysaccharides which may form viscous aqueous solutions.

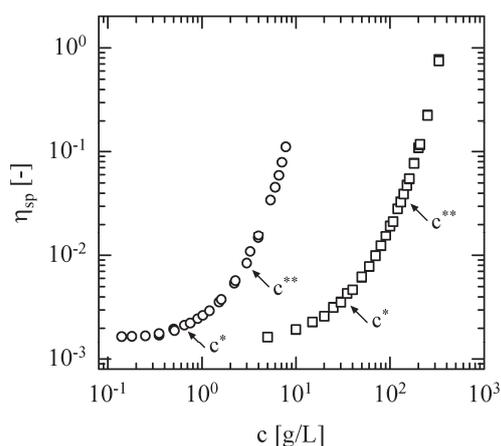


Figure 1. Specific viscosity (η_{sp}) vs. concentration from aqueous solutions of EPS_{ST-143} (circle) and dextran₅₀₀ (square). Arrows, coil overlap concentrations c^* , c^{**} .

The specific viscosity (η_{sp}), defined as $(\eta_{solution} - \eta_{solvent}) / \eta_{solvent}$, increased for both polysaccharides with their concentration, but significantly lower amounts of EPS_{ST-143} were necessary for a similar η_{sp} (Fig. 1).

From η_{sp} vs. c critical overlap concentrations (c^* , c^{**}), that describe the interactions between the polymer molecules, were extracted.^{5,6} η_{sp} increased only slowly until c^* , where the polysaccharides start to interact with each other. Then η_{sp} increased faster until at $c > c^{**}$ an entangled network was built.⁶ c^* and c^{**} were much lower for EPS_{ST-143} compared to dextran₅₀₀ (Table 1).

Table 1. Properties of EPS_{ST-143} and dextran₅₀₀.

	EPS _{ST-143}	dextran ₅₀₀
charge	neutral	neutral
M_m (Da)	3×10^6	5×10^5
monosaccharid ¹	Glc, Gal, Rha, GalN(Ac)	Glc
$[\eta]$ (mL/mg)	1.14	0.04
r_g (nm)	100	19
c^* (g/L)	0.68	41.50
c^{**} (g/L)	3.28	158.00

¹Glc, glucose; Gal; galactose; Rha, Rhamnose; GalN(Ac), N-Acetylgalactosamin.

The slopes of η_{sp} vs. c in the diluted region ($c < c^*$) were 0.8 and 1.3 for EPS_{ST-143} and dextran₅₀₀, respectively. They increased to 3.1 and 3.7 in the concentrated region ($c > c^{**}$). The data indicate that both polysaccharides behave as random coils in aqueous solutions.^{5,6}

The intrinsic viscosity $[\eta]$ as an indicator for the hydrodynamic volume occupied by a polymer in a diluted solution depends only on structure and molecular mass of the polysaccharide.⁷ EPS_{ST-143} with $M_m = 3 \times 10^6$ Da resulted in a higher $[\eta]$

(1.14 mL/mg) compared with dextran₅₀₀ ($[\eta] = 0.04$) with a lower M_m (5×10^5 Da). The radius of gyration (r_g , calculated from $[\eta]$ according to Tuinier et al., 1999⁸) is 5 fold lower for dextran₅₀₀.

Differences in $[\eta]$ may arise from M_m , but also from the more flexible chains and shorter branches of dextran₅₀₀ that allow a more compact shape.^{5,6,7} In contrast to that EPS_{ST-143} is a heteropolysaccharide with a more complex structure and stiffer molecules which occupy more space in solution. Hence, interactions between the EPS_{ST-143} molecules occurred at much lower concentrations compared to dextran₅₀₀.

Gelation of milk gels

The presence of the polysaccharides significantly affected G' , as a measure of gel stiffness, during the fermentation process (Fig. 2). In milk spiked with dextran₅₀₀ an earlier onset of gelation ($G' > 1$ Pa) occurred compared to reference samples without polysaccharide. Similar results were observed in chemically acidified gels after the addition of EPS_{ST-143}³ or dextran₅₀₀ (data not shown).

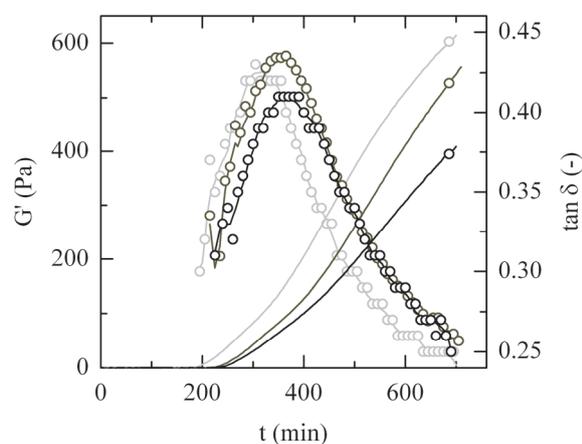


Figure 2. Development of gel stiffness (G' , lines) and $\tan \delta$ (open circles) during fermentation with DSM20259 and added polysaccharides. Non, black; EPS_{ST-143}, dark grey; dextran₅₀₀, light grey.

Both polysaccharides changed the gelation behaviour as is evident from $\tan \delta$ which describes the ratio of viscous to elastic contributions in a viscoelastic system. A local maximum of $\tan \delta$ between 300 and 400 min ($\tan \delta_{\max}$) occurred at the same time as G' exhibited a 'plateau' that indicated a temporary weakening in the gel structure.^{3,9} Higher values for $\tan \delta_{\max}$ in the presence of EPS_{ST-143} (0.433) and dextran₅₀₀ (0.435) compared to the reference (0.411) can be explained with more structural rearrangements in the protein network. Such changes of G' were observed in milk gels before with *in situ* produced EPS^{3,4}, added EPS^{4,10} or some commercially available polysaccharides (e.g. β -glucan)¹¹ and were more pronounced with increasing polysaccharide concentration³.

Impact of polysaccharides on milk gels

The gel stiffness at pH 4.6 (G'_{end}) was significantly higher in milk gels with added polysaccharides (Table 2). Similar values were observed for EPS_{ST-143} (556 Pa) and dextran₅₀₀ (587 Pa), whereas the concentrations used were complete different (EPS_{ST-143}, 0.25 g/kg; dextran₅₀₀, 5.0 g/kg). Apparently, both non-adsorbing polysaccharides induced depletion interactions, resulting in phase separation into a polysaccharide-rich phase and a protein-rich phase.² Hence, the interactions between the casein micelles are enhanced, which leads to faster aggregation and higher gel stiffness.^{2,3} Higher amounts of the compact and flexible dextran₅₀₀ molecules ($r_g = 19$ nm) were necessary to affect the aggregation of the casein micelles (~ 200 nm) compared to the larger and stiffer EPS_{ST-143} molecules ($r_g = 100$ nm).

Higher G'_{end} is often accompanied with lower syneresis (S)^{1,10} as it was observed in set gels with dextran₅₀₀. G'_{end} was 24 % higher and S was 44 % lower for milk gels with dextran₅₀₀ compared to the reference. However, after addition of EPS_{ST-143} whey separation in set gels increased. The larger

EPS_{ST-143} molecules seem to disturb the casein-casein interactions more than dextran₅₀₀, and thus such gels are more susceptible to syneresis upon applying an external force.

Table 2. Influence of added polysaccharides (PS) on set and stirred milk gels acidified with DSM20259.

PS	c _{PS} (g/kg)	set gel		stirred gel	
		G' _{end} ¹ (Pa)	S ² (%)	η _{app} ³ (Pa.s)	S ² (%)
-	-	447	15.3	136	39.4
EPS _{ST-143}	0.25	556	23.6	164	35.7
dextran ₅₀₀	5.00	587	8.6	176	36.6

¹G' at pH 4.6, ²syneresis after 1 d of storage at 6 °C, ³from flow curve at shear rate = 0.03/s.

In stirred gels syneresis decreased in the presence of EPS_{ST-143} and dextran₅₀₀. Furthermore, at low shear rate (0.03/s) an significant increase of the apparent viscosity (η_{app}) of gels with polysaccharides was observed. However, at 100/s, η_{app} decreased and was only slightly higher (EPS_{ST-143}, 0.33 Pa.s; dextran₅₀₀, 0.34 Pa.s) than for the reference (0.24 Pa.s). A defined stirring regime evenly distribute the polysaccharide molecules in the system and rearrangements of the casein aggregates during storage may lead to a more regular protein network in stirred milk gels compared to set gels.¹² The water binding properties of the dispersed polysaccharides increased the viscosity, on the one hand and lead to less whey separation on the other hand.

The differences of the molecular structure of the neutral polysaccharides dextran₅₀₀ and EPS_{ST-143} did not only influence the rheological behaviour in aqueous solutions, but also the gelation behaviour and the properties of the final milk gels. Further studies concerning the structure and the rheology of the two polysaccharides are necessary to gain a

deeper understanding of their behaviour in food products.

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REFERENCES

1. Tamime, A.Y., and Robinson, R.K. (1999), "Yoghurt: Science and Technology" Woodhead Publishing, USA.
2. Doublier, J.L., Garnier, C., Renarda, D., and Sanchez, C. (2000), "Protein-polysaccharide Interactions" *Curr. Opin. Colloid Interf. Sci.*, **5**, 202-214.
3. Mende, S., Peter, M., Bartels, K., Rohm, H. and Jaros, D. (2013), "Addition of purified exopolysaccharide isolates from *S. thermophilus* to milk and their impact on the rheology of acid gels" *Food Hydrocoll.*, **32**, 178-185.
4. Mende, S., Mentner, C., Thomas, S., Rohm, H., and Jaros, D. (2012), "Exopolysaccharide Production by Three Different Strains of *Streptococcus Thermophilus* and Its Effect on Physical Properties of Acidified Milk", *Eng. Life Sci.*, **12**, 466-474.
5. Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., and Price, J. (1981), "Concentration and Shear Rate Dependence of Viscosity in Random Coil Polysaccharide Solutions" *Carbohydr. Polym.*, **1**, 5-21.
6. Lefebvre, J., and Doublier, J. L. (2005), In "Polysaccharides: Structural diversity and functional versatility", 2nd ed., Marcel Dekker, New York, pp. 357 – 394.

7. Kulicke, W. M., and Clasen, C. (2010), "Viscosity of Polymers and Polyelectrolytes" Springer, Berlin.
8. Tuinier, R., Zoon, P., Olieman, C., Cohen Stuart, M.A., Fler, G.J., and de Kruif, C.G. (1999), "Isolation and physical characterization of an exocellular polysaccharide" *Biopolymers*, **49**, 1-9.
9. Lucey, J.A., and Singh, H. (1998), "Formation and Physical Properties of Acid Milk Gels: a Review" *Food Res. Int.*, **30**, 529-542.
10. Girard, M. and Schaffer-Lequart, C. (2007), "Gelation of Skim Milk Containing Anionic Exopolysaccharides and Recovery of Texture After Shearing" *Food Hydrocoll.*, **21**, 1031-1040.
11. Lazaridou, A., Vaikousi, H., and Biliaderis, C. G. (2008), "Impact of Mixed-linkage (1→3, 1→4) B-glucans on Physical Properties of Acid-set Skim Milk Gels" *Int. Dairy J.*, **18**, 312-322.
12. Corredig, M., Sharafbafi, N., and Kristo, E. (2011), "Polysaccharide-protein interactions in dairy matrices, control and design of structures" *Food Hydrocoll.* **25**, 1833-1841.

