Impact of Polysaccharides from Lactic Acid Bacteria on the Rheological Behaviour of Acid Milk Gels

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ABSTRACT

Polysaccharides of different origin are used as thickeners in dairy products to improve rheological and textural properties. Yogurt made with exopolysaccharide (EPS) producing starter cultures differ in gel stiffness, viscosity and syneresis. However, the interaction mechanisms between polysaccharides and milk proteins are not fully understood. Dextran from *Leuconostoc mesenteroides* ssp. and EPS from *Streptococcus thermophilus* ST-143 were added to milk prior to acidification, and the rheological properties of intact and stirred gels were characterised. Both polysaccharides influenced gelation properties and the rheology of the milk gels, however, a lower EPS amount is necessary to cause differences in gel stiffness.

INTRODUCTION

Exopolysaccharides (EPS) from lactic acid bacteria (LAB) are commonly used in the manufacture of fermented milk products such as yogurt and cheese to improve texture and sensory characteristics. Most studies deal with *in situ* produced EPS, whereas little is known about structure-function interactions between EPS and milk compounds. In many studies, no correlation between the produced amounts of EPS and the physical properties of the products was established.[1,2] The observed effects were mainly explained by chemical and structural characteristics of the polysaccharides.

The aim of our study was to analyze the effects of the addition of dextran, a well known polysaccharide, and EPS from a *S. thermophilus* strain on rheological and physical properties of chemically acidified milk gels and milk gels acidified by LAB. Chemically acidified milk is frequently used as model system for yogurt because it allows the investigation of milk gels without any influence of bacterial growth.

MATERIALS AND METHODS

Polysaccharides

Dextran from *Leuconostoc* ssp. (molar mass 500 kDa) was purchased from Sigma-Aldrich (Seelzen, Germany).

EPS used in this study was produced by *S. thermophilus* ST-143 (EPS_{ST-143}) during fermentation of a semi-defined medium (SDM)[3] with lactose in a 5 L bioreactor. Fermentation time was approx. 10 h at 40 °C, and pH was kept constant at 6.0 by adding 10 mol/L NaOH. Free EPS was purified using a cross flow filtration system (Sartorius AG, Göttingen, Germany) and precipitation with acetone. The amount of EPS in the freeze-dried powder was approx. 90 % as determined by the Dubois method.[4]

Preparation of milk gels

Reconstituted skim milk (dry matter 120 g/kg) was prepared by dissolving low heat skim milk powder (Alpavit Käserei Champignon Hofmeister GmbH & Co. KG, Lauben/Allgäu, Germany) in deionized

water. After 24 h storage the milk was heated to 91 °C for 15 min, subsequently cooled to 37 °C and spiked with dextran or EPS_{ST-143} prior to acidification. Microbially acidified milk gels (yogurt) were prepared by fermentation at 37 °C with a non EPS producing strain (*S. thermophilus* DSM20259 from Deutsche Sammlung für Mikroorganismen und Zellkulturen) or an EPS producing strain (*S. thermophilus* ST-143 from Chr. Hansen A/S). Chemical acidification was carried out after adding 3 % [w/w] glucono-δ-lactone (GDL) at 30 °C. pH during acidification was continuously monitored.

To characterise stirred products, the gels were subjected to a defined stirring regime using a perforated plate and a propeller stirrer. Prior to rheological measurements the samples were stored at 4 °C.

Rheological characterisation

Gelation of the milk gels was monitored with an ARES RFS3 rheometer (TA Instruments, Eschborn, Germany) with a concentric cylinder device. Strain was kept constant at $\gamma = 0.003$, and angular frequency was set to $\omega = 1$ rad/s. Flow curves were achieved by subjecting stirred milk gels of 15 °C to an upward shear rate ramp from 0 - 100 1/s within 100 s and to a corresponding downwards ramp; a plate-plate geometry (diameter: 25 mm, gap 1.3 mm) was used.

RESULTS AND DISCUSSION

Set milk gels

The addition of polysaccharides to milk before acidification changed the rheological behaviour of the resulting milk gels in dependance on the type and concentration of the polysaccharides (Table 1).

Adding 30 mg/g dextran almost doubled the stiffness of GDL gels (G' = 812 Pa) compared to the reference made without polysaccharides (G' = 463 Pa). A similar effect was observed in yogurt gels acidified by LAB. A much lower amount of EPS_{ST-143} was necessary to form gels with a similar G'. 0.15 mg/g EPS_{ST-143} were sufficient to form GDL gels which had similar stiffness (553 Pa) as gels with 5 mg/g dextran (GDL: 533 Pa, yogurt: 489 Pa). The further addition of EPS up to 0.45 mg/g resulted in a linear increase of G`(data not shown).

Table 1. Influence of added polysaccharides on the gelation parameters of set milk gels.

sample	c_{PS}[1] [mg/g]	t_{gel}[2] [min]	pH_{gel}[3] [-]	G'[4] [Pa]
GDL gel with dextran	0.00	29	5.12	463
	5.00	25	5.20	533
	12.50	23	5.26	695
	30.00	12	5.45	812
GDL gel with EPS_{ST-143}	0.15	25	5.34	553
Yogurt[5] with dextran	0.00	235	5.40	472
	5.00	215	5.41	489
	12.50	170	5.54	787
	30.00	155	5.76	937

[1] PS, polysaccharide; [2] time when G' > 1 Pa; [3] pH when G' > 1 Pa; [4] G' at the end of the fermentation: for GDL gels after 3 h (pH ~ 4.3) and for yogurt at a pH of 4.6. [5] produced with the non EPS producing strain *S. thermophilus* DSM20259

S. thermophilus ST-143 produced 0.16 mg/g EPS during yogurt fermentation. This amount resulted in gels with G`= 587 Pa, which is slightly higher than the stiffness of GDL gels with a comparable concentration of externally added EPS. In consistency with our results Girard & Schaffer-Lequart[3] and Kristo et al.[6] also reported a higher G' of milk gels with EPS.

Added polysaccharides did also change the gelation behaviour of the milk. At higher polysaccharide concentration, gelation started earlier and resulted in a higher pH_{gel}, which is defined as the pH when G' > 1 Pa.

Dextran and EPS_{ST-143} are uncharged polysaccharides with molar masses of approx. 5×10^5 Da and 4×10^6 Da, respectively. The addition of these neutral

polysaccharides to milk can cause phase separation resulting in polysaccharide-rich and protein-rich regions. Effective attraction through depletion mechanisms can occur between the casein micelles so that they can come closer to each other.[7] This may be the reason of the earlier onset of gelation at a higher pH. Structural differences between the polysaccharides may additionally affect the increased gel stiffness.[5] Polysaccharides with a high molar mass may enhance depletion interactions, and immobilize more protein-surrounding water, thus increasing the strength of the casein network.[8]

Stirred milk gels

Set gels were broken by applying a defined stirring regime and to allow proper rearrangements for rebuilding the structure stored overnight. However, compared with set gels the stirred gels are generally less stiff, and whey separation as measured in forced syneresis testing is enhanced. Stirred milk gels with polysaccharides present in the system have been shown to rearrange in less dense protein aggregates and a higher number of pores as compared to set gels was observed, which could explain differences in the rheological properties. [2,6,8]

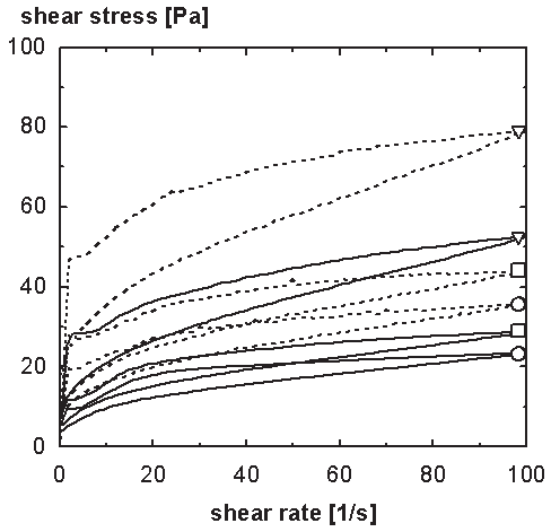

Figure 1. Flow curve (hysteresis loops) of stirred milk gels with dextran (circles: 0 mg/g; squares, 5 mg/g; triangles, 30 mg/g). Full lines, GDL gels, dashed lines, yogurt.

Figure 1 depicts flow curves of stirred GDL gels and yogurt containing dextran. The hysteresis loop area (A_H) between the upwards and downwards flow curve is related to structural breakdown during shearing and may also serve as an indicator for structure regeneration after cessation of shearing. Furthermore, Folkenberg et al.[9] pointed on an interrelation between the ropiness of EPS and A_H.

In stirred GDL gels, 5 mg/g dextran added to the base milk did not change hysteresis loop area significantly, but the apparent viscosity (η_A) at 100/s increased slightly. A_H and η_A were 353 ± 3 Pa/s and 239 ± 2 mPa.s, respectively, for gels without dextran. The highest dextran concentration applied in our study (30mg/k) resulted in the highest hysteresis loop area (663 ± 32 Pa/s) and the highest apparent viscosity (525 ± 3 mPa.s). Similar trends with increasing amounts of dextran were also achieved for microbially acidified gels, but A_H and η_A were generally higher for these systems compared with stirred GDL gels, presumably because of a reduced structural cohesion in chemically acidified milk.

A_H and η_A for yogurt with *in situ* produced $EPS_{ST\text{-}143}$ were 778 ± 22 Pa/s and 614 ± 1 mPa.s, respectively (data not shown). Higher η_A accompanied by a higher A_H appears to be typical for EPS producing starters.[2,10] A higher A_H indicates a lower ability of structure regeneration after shear-induced structure breakdown which could be explained with incompatibilities between proteins and polysaccharides.[9] However, the addition of similar amounts (0.15 mg/g) of EPS to GDL gels did not affect flow curves; changes were evident after an addition of 0.25 mg EPS/g A_H and η_A increase.

The ratio of hysteresis loop area to apparent viscosity describes the actual structure degradation. For all gels with dextran it was approx. 14, which indicates a stable gel structure independent from the amount of dextran added to the milk.

Syneresis of milk gels

Whey separation from stirred GDL gels and yogurt gels with different amounts of dextran after 1 day of storage at 4 °C ranged from 21 to 30 % and from 25 to 42 %, respectively (Figure 2). In general, chemically acidified milk gels appeared to be more stable and showed a higher water holding capacity compared with yogurt samples. Increasing amounts of dextran lowered syneresis to a different extent in dependence of the gel type. In yogurt, an amount of dextran as low as 5 mg/g already reduced whey separation, whereas in stirred GDL gels, significant effects were recognized not until a concentration of 30 mg/g.

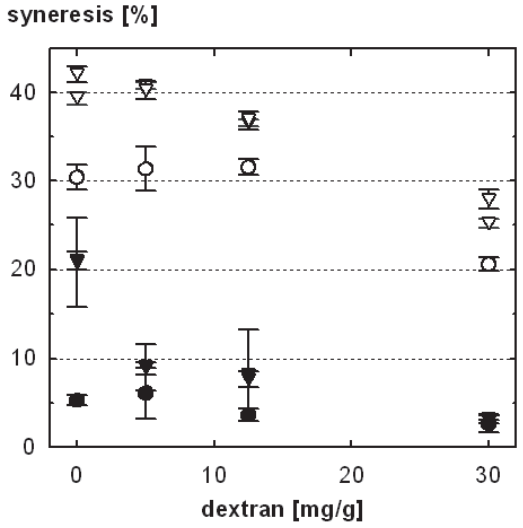

Figure 2. Syneresis of chemically and microbially acidified milk gels with dextran after 1 day storage at 4 °C. Open symbols, stirred gels; closed symbols, set gels; Circles, GDL gels; Triangles, yogurt gels.

Although a similar trend was observed, forced syneresis of set milk gels was generally lower than that of stirred gels.

Dextran presumably binds water inside the pores of the gel network and therefore reduces syneresis. Furthermore, the microstructure of gels is changed through the addition of polysaccharides and this partly affects susceptibility to syneresis.

The addition of dextran and a well-purified EPS influenced set and stirred milk gels. These results are different to investigations, that found the addition of semi-purified EPS less effective.[11,12] Further work will be conducted to gain a better insight into the characteristics of milk gels with added exopolysaccharides.

ACKNOWLEDGMENTS

We wish to thank Chr. Hansen A/S for providing their strain.

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