Addition of purified exopolysaccharides from lactic acid bacteria affects the rheological behaviour of acid milk gels

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ABSTRACT

The exact mechanism behind the structure-function interaction between exopolysaccharides (EPS) produced from lactic acid bacteria during fermentation and milk proteins are still elusive. Yogurt productions with various starter cultures revealed large differences in gel stiffness, viscosity and EPS amount. To eliminate the impact of starter and fermentation parameters on texture properties, EPS from two strains (one ropy, one capsular) were purified and added to milk. Subsequent gelation experiments with a chemical acidifier clearly demonstrated that both EPS increased gel stiffness, but that lower amounts of ropy EPS were necessary for comparable gel stiffness.

INTRODUCTION

Exopolysaccharid (EPS)-producing lactic acid bacteria (LAB) are widely used for the manufacture of dairy products to improve texture and to decrease syneresis without the addition of hydrocolloids. Whereas in many studies the texture-enhancing effect of *in situ* produced heteroexopolysaccharides has been confirmed, little is known about the exact mechanisms of the structure-function interaction between these natural thickeners with other milk constituents. The aim of our study was to evaluate the effect of external addition of EPS on physical and rheological properties of model acid gels and yogurt.

MATERIALS AND METHODS

Commercially available UHT-milk (dry matter: 92.7 g/kg, fat: 1 g/kg) was fortified to 120 g/kg dry matter with low heat skim milk powder (Alpavit Käserei Champignon Hofmeister GmbH & Co. KG, Lauben/Allgäu, Germany) and demineralised water. Four single strain starters and two combined yogurt cultures (Table 1) were used for fermentation which, depending on the type of the measurement, was performed in glass vials, plastic cups, or in situ in a concentric cylinder device of an ARES RFS3 rheometer (TA Instruments, Eschborn, Germany) at 43 °C. Strain was kept constant at $\gamma = 0.003$, and angular frequency was set to $\omega = 1$ rad/s. pH during acidification was continuously monitored. For subsequent measurement of apparent viscosity, the gels were subjected to a defined stirring procedure using paddle and propeller stirrers. Flow curves were achieved by subjecting stirred yogurt of 15 °C to an upward shear rate ramp from 0 – 100 1/s within 100 s and to a corresponding downward ramp; a cone-and-plate geometry (diameter: 25 mm, cone angle: 0.04 rad) was used.

In another set of experiments, fortified skim milk was acidified by adding 3 % glucono-δ-lactone (GDL). Prior to acidification at 30 °C, commercial dextran or EPS isolated as described below were added to the base milk at various levels.

EPS from *S.thermophilus* ST-143 (EPS_{ST-143}) and from *S.thermophilus* DSM 8713 ($EPS_{DSM8713}$) were produced by fermenting a semi-defined medium (SDM)[1] and whey permeate in a 2 L bioreactor (Applikon® Biotechnology, Schiedam, The Netherlands), respectively. Fermentation time was approx. 10 h, temperature was 40 °C, and pH was kept constant at 6.0 by adding 10 mol/L NaOH. After removing bacteria cells by centrifugation, EPS were concentrated by ultrafiltration, isolated by a combined treatment with acetone and trichloracetic acid, purified by dialysis against water, and freeze-dried. The amount of EPS in the freeze-dried powder was determined by the Dubois method.[2]

RESULTS AND DISCUSSION

Biological acidification

The amount of EPS produced *in situ* during yogurt production largely varied for the selected strains, as did the storage modulus at pH = 4.6 (Table 1).

Table 1. Stiffness and EPS content of yoghurt made with different strains.

Starter[1]	Type[2]	G' (Pa) at pH=4.6	EPS[3] (mg/kg)
DSM 8713	S	335	40
ST-143	S	334	46
DGCC 7710	S	268	109
DGCC 7785	S	390	8
YO-MIX 410	S+L	166	130
YO-MIX 532	S+L	239	94

[1]DSM 8713, from Deutsche Sammlung für Mikroorganismen und Zellkulturen; ST-143 and YO-MIX from Danisco A/S, other, from Chr. Hansen A/S
[2]S, *S.thermophilus*; S+L, *S.thermophilus* + *L.delbrueckii* ssp. *bulgaricus*
[3]in glucose equivalents

A direct relationship is troublesome, because of other factors of influence, e.g. fermentation time, which widely differed (275 - 790 min). Interestingly, we found a high concentration of EPS in yoghurt from YO-MIX 532, which has been declared as EPS^- by the provider, but no increase in viscosity of the stirred product. This confirms the fact that, e.g. composition, charge, branching and molecular weight are more important for structure-enhancing effects than the actual amount.

Although ST-143 (ropy EPS) and DSM 8713 (capsular EPS) produce different types of polysaccharides, the resulting set-style yogurts exhibited similar properties. On the other hand, stirred yogurt made with these strains showed a completely different rheological behaviour (Fig. 1).

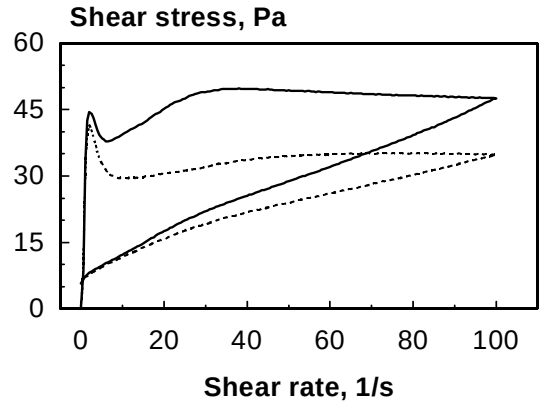

Figure 1. Shear stress-Shear rate profiles of stirred yogurt from two productions with DSM 8713 (dotted line) and ST-143 (full line). Average curves calculated from n = 4.

Hysteresis loop areas between the upward and downward flow curve, which correspond to the amount of structure degradation at a defined shear regime[3] were 1839 ± 131 and 1037 ± 30 Pa/s for ST-143 and DSM 8713, respectively. Folkenberg *et al.* (2006)[4] found a strong correlation between non-orally determined ropiness and hysteresis loop area and explained the impaired reassociation of gel particles with the incompatibility between casein and EPS. Additionally, lower values for syneresis (centrifugation method) were detected when ST-143 was used for fermentation (data not shown).

Chemical acidification

There are two major drawbacks, when studying the structure-enhancing properties of purified EPS from LAB: their low yield and the difficulty to find a suitable control starter culture. Therefore, we used GDL as chemical acidifier for reproducible gelation experiments in small-scale experiments. Different amounts of EPS_{ST-143} and $EPS_{DSM8713}$ were added to the milk and G' was monitored during subsequent acidification (Fig. 2). Gel stiffness after 3 h (pH approx. 4.2) was close to G' of the reference sample after addition of 0.10 g/kg $EPS_{DSM8713}$, whereas the same concentration of EPS_{ST-143} resulted in a marked increase of approx. 38 %. To achieve a similar gel stiffness 0.25 g/kg of $EPS_{DSM8713}$ were necessary. A further increase of EPS_{ST-143} (0.25 g/kg) resulted only in a small increase of gel stiffness, suggesting that incompatibilities between proteins and polysaccharides are beginning to take effect.

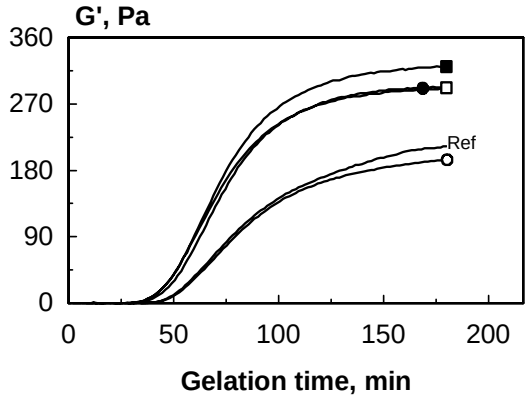

Figure 2. Development of G' of UHT-milk (12 % w/w) acidified with 3 % GDL at 30 °C. Ref, reference sample without EPS; Closed symbols, addition of EPS_{ST-143}; Open symbols, addition of $EPS_{DSM8713}$. Squares, 0.25 g/kg EPS; circles, 0.10 g/kg EPS.

Much higher concentrations were necessary for dextran (0.5 and 1.0 g/kg) to cause an increase of G' of 40 and 68 %, respectively (data not shown). There are only very few studies, which try to compare the impact of *in situ* and added EPS on the rheological properties of milk gels.[5,6] The common conclusion is that *in situ* production is a better approach than adding EPS, but the authors did not investigate the relationship between milk proteins and polysaccharides to a greater extent. Additional drawbacks in these studies are the low purities of the prepared EPS samples and the use of completely different starter cultures for EPS^- and EPS^+ comparison. Hassan et al.[7] made an interesting approach with a mutant strain of an EPS producer, which lost its ability of EPS production. Studying microctructure and rheology of yogurt manufactured with these strains, Hassan et al.[7] concluded that stirring affects yogurt with EPS differently. Experiments with stirred model gels with added EPS are under progress.

ACKNOWLEDGMENTS

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