



# Microstructure and stability of skim milk acid gels containing an anionic bacterial exopolysaccharide and commercial polysaccharides



Sandra I. Laneuville, Sylvie L. Turgeon\*

Dairy Research Center STELA, Institute of Nutrition and Functional Foods, Faculty of Agriculture and Food Science, Université Laval,  
2425 rue de l'Agriculture, Pavillon Paul-Comtois, Québec G1V 0A6, Canada

## ARTICLE INFO

### Article history:

Received 26 June 2012

Received in revised form

22 January 2014

Accepted 23 January 2014

## ABSTRACT

The structure and stability of acid milk gels containing an anionic exopolysaccharide (EPS) produced by *Lactobacillus rhamnosus* RW-9595M in combination with different commercial polysaccharides were studied. Gel internal structure was visualised using confocal scanning laser microscopy; protein, EPS and polysaccharides were labelled using different fluorophores to resolve their specific location. EPS at 0.01% resulted in lower syneresis (2.5%) than control (3.7%); micrographs showed EPS co-localised with protein and reinforcing network connectivity. However, 0.05% EPS increased syneresis to over 17%, although no significant changes in gel strength were detected. Pectin or agar–agar alone at 0.05% resulted in very low syneresis (<1.8%). Both polysaccharides were found to form a tenuous secondary yet more ramified network, effectively reducing pore size. Mixing pectin or agar–agar with EPS at 0.05% resulted in a loss of their functionality. Anionic polysaccharides with stiff molecular structures, such as xanthan or  $\kappa$ -carrageenan, induced extensive protein self-aggregation and syneresis.

© 2014 Elsevier Ltd. All rights reserved.

## 1. Introduction

The majority of lactic acid bacteria are recognised to produce exopolysaccharides (EPSs) to varying degrees, amounts below 150 mg L<sup>-1</sup> are typical, but values ranging from 25 to 2000 mg L<sup>-1</sup> depending on the strain and incubation conditions have been reported (Lucey, 2004; Robijn, 2006; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). The presence of an EPS can have substantial and diverse effects on the stability, viscoelastic, textural and organoleptic properties of acid milk gels and fermented milk products including yoghurt (Folkenberg, Dejmeke, Skriver, & Ipsen, 2005; Girard & Schaffer-Lequart, 2007b) and cheese (Dabour, Kheadr, Fliss, & LaPointe, 2005; Hassan, Awad, & Muthukumarappan, 2005). The differences in the effects imparted have been related to differences in the molecular properties of the EPS, including the molecular weight (Faber, Zoon, Kamerling, & Vliegthart, 1998; Lazaridou, Vaikousi, & Biliaderis, 2008), ropy versus non-ropy or capsular versus non capsular character (Dabour et al., 2005; Hassan, Corredig, & Frank, 2002a), molecular conformation, chain stiffness (rigid rod versus random coil), type of linkages (Kleerebezem et al., 1999; Lazaridou et al., 2008) and the presence

of charged groups (Folkenberg et al., 2005; Girard & Schaffer-Lequart, 2007b). Lower syneresis has been reported numerous times in both types of products (Amatayakul, Sherkat, & Shah, 2006; Doleyres, Schaub, & Lacroix, 2005; Folkenberg et al., 2005; Guzel-Seydim, Sezgin, & Seydim, 2005; Hassan, Frank, & Qvist, 2002b; Hassan, Ipsen, Janzen, & Qvist, 2003; Robitaille et al., 2009).

In yoghurt, the desired functionality for the added polysaccharides differs for stirred to set style yoghurts. In general, the presence of polysaccharides or EPS, besides affecting syneresis, can also result in higher viscosities in stirred yoghurts (Doleyres et al., 2005; Hassan et al., 2003); whereas lower moduli values ( $G'$  and  $G''$ ) and higher elastic character have been reported in set style yoghurts (Hassan et al., 2003). In most acid milk products, the presence of polysaccharides, either added or produced in situ, results in the formation of denser protein networks as observed by confocal and electron microscopy (Hassan et al., 2002b, 2003). It has also been reported that the variability often found in gel structure following heat treatment, which is usually brought about by differences in the initial pH of milk, can be reduced by the presence of polysaccharides. The latter appears to influence the arrangement of the protein clusters and the size of the pores within the gel network (Oh, Wong, Pinder, Hemar, & Anema, 2007b).

The molecular charge density of a polysaccharide has been shown to play an important role in determining its functionality in acid milk gels (Girard & Schaffer-Lequart, 2007b). Neutral

\* Corresponding author. Tel.: +1 418 656 2131x4970.

E-mail address: [Sylvie.Turgeon@fsaa.ulaval.ca](mailto:Sylvie.Turgeon@fsaa.ulaval.ca) (S.L. Turgeon).

polysaccharides, such as  $\beta$ -glucan, starch or guar, do not directly participate in the protein network and tend to separate into the serum phase in the pores (Hassan et al., 2003). They act as fillers by absorbing the water in the pores, increasing the viscosity of the serum phase, rendering it less prone to flow, thus resulting in decreased syneresis (Dabour et al., 2005; Hassan et al., 2003; Lazaridou et al., 2008; Oh, Anema, Wong, Pinder, & Hemar, 2007a; Pleijsier, de Bont, Vreeker, & Ledeboer, 2000; Robitaille et al., 2009).

On the other hand, anionic polysaccharides like xanthan gum or low methoxyl pectin (LM-pectin) can interact directly via electrostatic attractive interactions with the aggregating micelles, and have different effects on the properties of the product (Girard & Schaffer-Lequart, 2007b; Low et al., 1998; Sanchez, Zuniga-Lopez, Schmitt, Despond, & Hardy, 2000). Depending on the specific molecular characteristic of the EPS or polysaccharide (e.g., charge density, chain stiffness), they can assist gelation and stabilise the protein network by taking part in it (Pleijisier et al., 2000; Sanchez et al., 2000). The reported lower  $G'$  values in set type yoghurts containing EPS (Guzel-Seydim et al., 2005; Rawson & Marshall, 1997) have been related in some cases to the disruption brought about by strong protein–polysaccharide interactions or depletion interactions occurring above the isoelectric point (pI) of the protein. Other authors propose that charged EPS (anionic EPS) increases gel firmness ( $G'$ ) by forming bridges between neighbouring protein aggregates (Girard & Schaffer-Lequart, 2007b; Pleijsier et al., 2000). However, if excessive amounts of EPS or polysaccharide are added, non-adsorbing neutral polysaccharides can demix due to polymer incompatibility or to depletion flocculation interactions (Sanchez et al., 2000; Tuinier, ten Grotenhuis, & de Kruijff, 2000), resulting in increased syneresis. Whereas an incompatibility scenario can also occur with anionic polysaccharides at pHs above the pI of the proteins, where the polysaccharide can influence protein flocculation/aggregation and have an important impact on the final structure of the gel.

Acid milk gels containing a variety of commercial polysaccharides have been studied (Acero-Lopez, Alexander, & Corredig, 2010; Brennan & Tudorica, 2008; Everett & McLeod, 2005; Fagan, O'Donnell, Cullen, & Brennan, 2006; Keogh & O'Kennedy, 1998; Oh et al., 2007a). However, to our knowledge, no studies have aimed at understanding the effect of adding a commercial polysaccharide to a system where an EPS is present. Furthermore, we were interested in studying charged EPS and polysaccharides since they can electrostatically interact with the protein matrix (attraction) and with each other (repulsion) providing further complexity to such mixed systems.

In this study, skim milk gels were allowed to form by acidification using glucono- $\delta$ -lactone (GDL) as a model for set-style yoghurt products. The choice of commercial polysaccharides was based on commonly employed ingredients in the Canadian dairy industry, which include pectin, xanthan and  $\kappa$ -carrageenan (as anionic polysaccharides), agar–agar (very weakly charged) and modified cornstarch (neutral polysaccharide). To control the amount of EPS present in the system, it was added as a bioingredient. Previous studies have shown that adding an EPS as a bioingredient may produce somewhat different results compared with an EPS produced in situ. Namely, the consistency index of yoghurts containing EPS was higher (thicker texture) than controls; however, the highest value was obtained when the EPS was produced in situ (Doleyres et al., 2005). It is noteworthy that Doleyres et al. (2005) utilised a partially purified EPS and this may have had an effect on their results. The approach followed in this study allowed identifying main interactions that occur when EPS and commercial polysaccharides are present in acid milk products.

## 2. Materials and methods

### 2.1. Materials

Skim milk powder (SMP) was graciously supplied by Agropur, Granby, Canada: RIMO low heat, with 35.0% protein (29.4% casein), 48.7% sugars (lactose), 10.0% ashes; and 6.3% moisture. The EPS studied was produced by *Lactobacillus rhamnosus* RW-9595M during repeated-batch culture with immobilised cell technology, which allowed a production exceeding 1.7 g EPS per litre (Bergmaier, Champagne, & Lacroix, 2003). The EPS was kindly provided in its purified form, following the procedure presented in Van Calsteren, Pau-Roblot, Bégin, and Roy (2002), by Prof. Denis Roy (INAF, Laval University, Quebec, Canada). The structure of this EPS has been previously determined (Van Calsteren et al., 2002) and consists of an heptasaccharide repeating unit containing rhamnose and glucose linked by  $\alpha(1\rightarrow3)$  or  $\alpha(1\rightarrow2)$  links in the main chain, and a side chain composed of 1 galactose and 1 pyruvate residue, the latter being responsible for its anionic character. Since this particular EPS forms coacervates instead of fractal aggregates with milk proteins (Turgeon & Laneuville, 2009) and bears  $\alpha$  linkages, it is considered to have a flexible molecular structure based on the concepts presented by Fellows and Doherty (2005) and Kleerebezem et al. (1999).

Commercial polysaccharides included LM-pectin (95.7% solids, 92.6% total carbohydrate, 1.4% minerals, 4.3% moisture, CP Kelco, Lille Skensved, Denmark);  $\kappa$ -carrageenan (type III from *Eucheuma cottonii*; 91.7% total solids, Sigma Chemicals, St-Louis, MO, USA); Xanthan gum (food grade Keltrol RD, 96.4% total carbohydrate, 3.0% protein, CP Kelco, Chicago, IL, USA); agar–agar (88.4% solids, Tic Gums, Belcamp, MD, USA); and modified cornstarch from Waxy Maize, hereafter referred to as “starch” (Thermflo, 90.7% solids, 90.1% total carbohydrate, 0.5% minerals, National Starch, Brampton, Canada).

### 2.2. Acid milk gel preparation

Skim milk acid gels were prepared to a final total solids concentration of 12%. This concentration is lower than the one typically employed for yoghurt manufacture, which ranges from 14 to 16%. It is known that increasing milk solids can decrease syneresis (Amatayakul et al., 2006; Lazaridou et al., 2008) and it was considered that at 12% solids, the effect of the added polysaccharides would be exacerbated. For sample preparation, skim milk powder (SMP) was dispersed at 15% (w/w) and polysaccharides were dispersed at 0.6% (w/w) in deionised water, both were mixed for 1 h and allowed to hydrate overnight at 4 °C, excepting for  $\kappa$ -carrageenan, agar–agar and starch, which were prepared 1 h prior to mixing since these polysaccharides require heat to dissolve. If needed, the pH of the polysaccharide solutions was adjusted to  $6.65 \pm 0.03$  using 1 M NaOH and/or 1 M HCl, care was taken to avoid concentration changes during heating or pH adjusting.

Dispersions were allowed to regain ambient temperature before mixing, then 0.01, 0.025 or 0.05% (w/w) polysaccharide or EPS + polysaccharide were added to the skim milk dispersion (e.g., samples prepared with EPS + polysaccharide at 0.05% contained 0.025% EPS and 0.025% polysaccharide). Total solids were adjusted to 12% by adding the appropriate amount of deionised water (w/w) and the mixtures were allowed to stir for an additional 30 min. A heat treatment of 90 °C for 2 min was applied in a water bath under continual stirring (temperature measured at the core of the sample, total heating time to reach the target temperature was  $\sim 8.5$  min); samples were cooled to  $\sim 30$  °C in a water/ice bath (cooling time  $\sim 2$  min). Then, 0.02% NaNO<sub>3</sub> was added as a preservative and

Download English Version:

<https://daneshyari.com/en/article/2434364>

Download Persian Version:

<https://daneshyari.com/article/2434364>

[Daneshyari.com](https://daneshyari.com)