Food Hydrocolloids 23 (2009) 1299–1304

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Effect of protein supplementation on the rheological characteristics of milk permeates fermented with exopolysaccharide-producing *Lactococcus lactis* subsp. *cremoris*

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ARTICLE INFO

Article history:
Received 30 December 2007
Accepted 12 November 2008

Keywords:
Whey proteins
Exopolysaccharides
Lactococcus lactis subsp. *cremoris*
Milk permeate
Protein–exopolysaccharide interactions

ABSTRACT

This research studied the effect of addition of whey proteins on the rheological properties of ultrafiltration permeate fermented with the exopolysaccharide (EPS)-producing strain *Lactococcus lactis* subsp. *cremoris* JFR1. Milk permeates containing 8% solids and various levels of added whey proteins (0, 2, 4, 6 and 8%) were fermented for 12 h at 30 °C. The rheological properties of the fermented samples were then evaluated and compared to controls fermented with a non-EPS producing strain. Scanning electron microscopy was also employed to confirm the existence of interactions between whey protein aggregates and EPS. The presence of EPS considerably increased the viscosity and viscoelastic properties of the media, especially in samples containing >2% whey protein added. The results obtained demonstrate the importance of EPS–protein interactions in structure formation and may help explain the viscosifying mechanism of EPS in fermented dairy products. Production of highly viscous material could potentially be employed in the future as a novel fiber-rich functional ingredient in dairy products.

1. Introduction

Exopolysaccharides (EPS) are defined as long chain polysaccharides secreted by microbial cells (Laws, Gu, & Marshall, 2001). They are commonly associated with the 'slimy' or 'ropy' characteristic of some acidified milk products. Exopolysaccharides produced by some strains of lactic acid bacteria (LAB) have been found to improve textural properties and increase water retention in fermented dairy products (Cerning, Bouillanne, Desmazeaud, & Landon, 1986; Ruas-Madiedo, Tuinier, Kanning, & Zoon, 2002). The presence of EPS seems to contribute to texture, mouthfeel, taste perception and stability of the final products (Folkenberg, Dejmek, Skriver, & Ipsen, 2005; Girard & Schaffer-Lequart, 2007a; Hassan, Frank, Schmidt, & Shalabi, 1996). In addition to processing functionality, the consumption of EPS has been associated with health benefits (Dal Bello, Walter, Hertela, & Hammesa, 2001; Kitazawa et al., 1998; Korakli, Ganzle, & Vogel, 2002). Therefore, the development of EPS produced by LAB as a functional ingredient in foods has great potential.

The yield of EPS produced by different LAB is generally quite low. It has been reported to vary widely from 50 to 2700 mg/L (Macedo, Lacroix, Gardner, & Champagne, 2002). Several attempts have been made to control the fermentation conditions to increase EPS production (Grobben et al., 1998; Macedo et al., 2002), but there are still many unresolved questions. Because of their low production yields, very few studies have been carried out on the application of EPS produced by LAB as a functional ingredient, and most research has focused on the structuring effect of the polysaccharide *in situ*, mainly for the manufacture of cheese and yogurt (Folkenberg et al., 2005; Hassan, Frank, & Elsoda, 2003; Hassan et al., 1996). In these systems, fermentation time is relatively short, and small amounts of EPS are present in the final product. The utilization of a fermented medium as such, without performing EPS isolation, as a functional ingredient, has yet to be fully explored.

Multiple studies have reported an effect of the protein and carbohydrate composition of the fermentation media on the production of EPS by LAB (Grobben et al., 1997; Marshall, Cowie, & Moreton, 1995; Petry et al., 2003). The EPS production seems to increase with increasing protein content in the medium (Grobben et al., 1998). Other medium components such as minerals, specific amino acids, and carbohydrates can also affect the composition and the yield of EPS (De Vuyst & Degeest, 1999; Grobben et al., 1998). It has been also suggested that the balance between the carbon and nitrogen contents is fundamental to achieve high EPS yields (De Vuyst & Degeest, 1999; De Vuyst, Vanderveken, Van de Ven, & Degeest, 1998).

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doi:10.1016/j.foodhyd.2008.11.004

Milk, whey or their processing byproducts are suitable substrates for production of EPS because of their high lactose content. Macedo et al. (2002) developed a whey permeate-based medium for the production of EPS by *Lactobacillus rhamnosus* RW-9595 M, and studied the effect of different nitrogen sources, salts, amino acids and vitamins on EPS production. The most significant increases in EPS production were achieved with the addition of salts and a strong interaction was reported between the effects of protein and salts.

Although the rheological properties of various dairy products containing EPS have been reported (Girard & Schaffer-Lequart, 2007a; Hassan et al., 1996; Laws et al., 2001; Rawson & Marshall, 1997; Torre, Tamime, & Muir, 2003), the mechanisms that generate the observed changes in texture remain unclear. It has been hypothesized that interactions between proteins and polysaccharides are responsible for the changes in the texture of milk fermented by EPS-producing cultures (De Kruif & Tuinier, 1999; Girard & Schaffer-Lequart, 2007a; Tuinier, Dhont, & De Kruif, 2000) but the specifics of these interactions are yet not fully understood.

Interactions of some EPS (specially anionic ones) with whey proteins in model systems have been previously studied (Ayala-Hernandez, Hassan, Goff, Mira de Onduna, & Corredig, 2008; De Kruif & Tuinier, 1999; Girard & Schaffer-Lequart, 2007b; Tuinier et al., 2000). However, the importance of these interactions for structure formation in the fermented medium has still not been fully evaluated. The objective of the present experiments was to acquire greater understanding of the conditions that favor changes in the rheological properties of permeate samples fermented with *Lactococcus lactis* subsp. *cremoris* producing EPS, and better control structure formation.

This EPS is mainly composed of glucose (60%), rhamnose and galactose in an average ratio of approximately 4:2:1; it has a molecular mass of about 2×10^6 Da and a radius of gyration of 70 nm (Ayala-Hernandez, Hassan et al., 2008; Ayala-Hernandez, Goff et al., 2008). It has also been reported that this polysaccharide interacts with whey proteins, when the whey proteins are attached to colloidal particles (Ayala-Hernandez, Hassan et al., 2008; Ayala-Hernandez, Goff et al., 2008), suggesting the presence of negative charges. A better understanding of the conditions that favor the interactions of EPS with milk proteins would help in optimizing its utilization as a functional ingredient in dairy products. For this purpose, milk permeate with a higher concentration of lactose and whey protein contents was fermented by the ropy strain *L. lactis* subsp. *cremoris* JFR1. The rheological properties of the fermented media were evaluated and compared with those of samples fermented with a non-EPS producing strain.

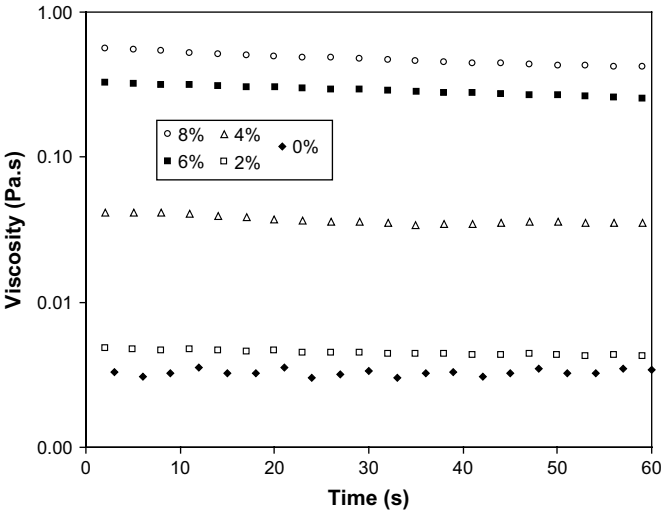

Fig. 2. Viscosity measured at a constant rate of 100 s^{-1} of the fermented milk permeates containing different protein concentrations as a function of time. Measurements were carried out at 4 °C.

2. Materials and methods

2.1. Concentrated milk permeate

Milk permeate with a higher lactose concentration was prepared in the pilot plant facility of Parmalat R&D Canada (London, ON, Canada). The original milk permeate was obtained by ultrafiltration of milk and then concentrated using a reverse osmosis system (pressure driven membrane process that separates molecules of less than 100 Da), obtaining a final product of approximately 8% solids. This permeate contained minimal amounts of protein (0.02% determined by Dumas combustion method in a FP-2000 protein analyzer, Leco Corporation, Saint Joseph, MI, USA).

2.2. Fermentation media

Samples of milk permeate were prepared with different protein contents: 0, 2, 4, 6, 8% (w/v) by adding research grade whey protein

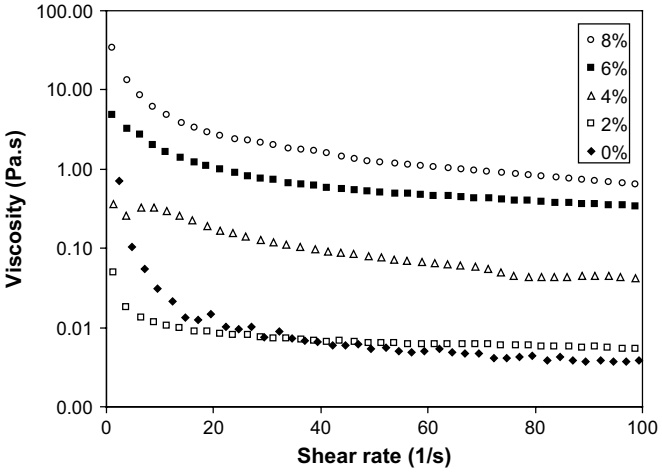

Fig. 1. Viscosity of fermented milk permeate containing different protein concentrations as a function of shear rate. Measurements performed at 4 °C.

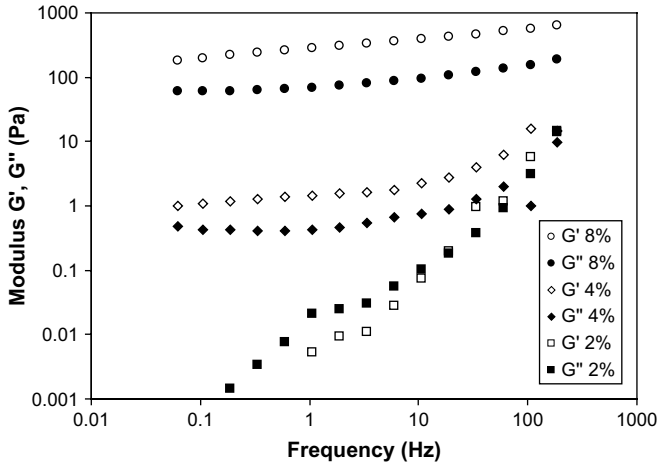

Fig. 3. Storage (G′) and loss (G″) modulus of fermented milk permeates with different protein contents presented as a function of frequency. Measurements performed at 4 °C.