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Molar mass and rheological characterisation of an exopolysaccharide from *Pediococcus damnosus* 2.6

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Abstract

The molar mass and rheological properties of an exopolysaccharide (EPS) from *Pediococcus damnosus* 2.6 were investigated. The molar mass was determined by asymmetrical flow field-flow fractionation coupled with multiangle light scattering and refractive index detection. The EPS was observed to be a flexible chain polymer with a molar mass value of 4×10^6 g mol^{-1}. Heating the sample at 80 °C for 10 min caused a shift to lower hydrodynamic radius. The rheological behaviour of the EPS was compared to that of a commercial cereal β-glucan (0.359×10^6 g mol^{-1}). The maximum storage modulus, G'_{max} for EPS solution was lower than that for the cereal β-glucan at all concentrations, while the relaxation time, $t_{G'=G''}$ was higher. The G'_{max} was reduced on heating the EPS solution at 80 °C for 10 min, likely indicating some conformational changes. Three-dimensional models of the polymers revealed some differences in intramolecular hydrogen bonds. The EPS molecule had a ropy nature in solution and this could make it suitable for usage as a thickener in food systems.

Keywords: Exopolysaccharide; *Pediococcus damnosus* 2.6; Molar mass; Root mean square radius; Polydispersity; Loss modulus; Storage modulus

1. Introduction

There has been a rapid growth in the number of studies on the structure and rheology of biopolymer solutions. Work on food gels additives is initiated by the growth in perception of healthy diets and in the design of low-fat spreads and desserts (Ross-Murphy, 1995). Most of the food thickening and gelling agents widely used in the food industry today are polysaccharides such as guar gum, pectin, locust bean gum and starch from plants; gelatin from animals; alginate and carrageen from seaweed; gellan gums and xanthan from bacteria (Vandamme, Bruggerman, De-Baets, & Vanhooren, 1996).

The *in situ* production of polysaccharides by GRAS (generally regarded-as-safe) microorganisms in food products is increasingly drawing the attention of the food industry and consumers. The most common exopolysaccharides-producing bacteria today are the lactic acid bacteria (Cerning, 1995). Exopolysaccharides (EPS) from lactic acid bacteria are either attached to the cell wall or secreted into the extracellular environment (Cerning, 1990), and can enhance the rheological properties of the final product (Perry, Mahon, & Oberg, 1997; Sebastiani & Zelger, 1998). One of such EPS (a straight-chained β-(1 → 3) glucan structure with β-(1 → 2) glucose branches), was isolated by Dueñas-Chasco et al. (1997) from *Pediococcus damnosus* 2.6. It was found to thicken the consistency of cider in the Basque Country. Interestingly, yoghurts with EPS-producing strains have demonstrated less shear-thinning behaviour as compared to those with non-EPS strains (Sutherland,

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1977). The *in situ* production of thickeners could improve the texture, viscosity, sensory, and nutritional properties in food products (Cerning, 1995; Ricciardi, Parente, & Clementi, 1994).

Mårtensson et al. (2000) used viscosity measurements to monitor EPS production from *Pediococcus damnosus* 2.6 in a fermentation medium. This polymer has been observed to be responsible for the ropy consistency in the final product (Mårtensson, Dueñas-Chasco, Irastorza, Öste, & Holst, 2003). Furthermore, studies on EPS from *Pediococcus damnosus* 2.6 in an oat-based medium, have shown some positive physiological effects (Lambo-Fodje, Öste, & Nyman, 2006; Mårtensson et al., 2005). However, nothing is known about the molar mass and the rheological properties of this polymer. This work was aimed at studying the molar mass and viscoelastic properties of the EPS from *Pediococcus damnosus* 2.6 (Pd). The rheological properties of a commercial cereal β-glucan were also measured as a comparison.

2. Materials and methods

The commercial cereal β-glucan (molar mass 0.359×10^6 g mol^{-1}) was obtained from Megazyme International, Co. Wicklow, Ireland.

2.1. EPS purification

The crude EPS from Pd was provided by the Division of Applied Microbiology, Lund University. The production and isolation procedures have been described in previous studies (Paese, 2003). The polysaccharide was received as a brownish-white powder (~50% pure) and contained impurities such as lactic acid (~5%), glucose (~25%) and acetone (~20%), related to the bacterial origin of the polysaccharide, the growth medium and the solvent used for isolation. In order to further purify the EPS, the crude material was dissolved in distilled water and dialyzed against distilled water (5 L) for 24–36 h, changing the water twice daily. The dialysis tubing used had a molar mass cut-off of 500 Da (Spectrum laboratories Inc., Rancho Dominguez, CA), The dialysates were lyophilised and weighed. The EPS obtained had a purity of ~90% according to NMR and GLC analyses.

2.2. EPS characterisation

The monomeric composition of the purified EPS was analysed as their alditol acetates by gas–liquid chromatography (GLC) on a DB-225 column (J&W Scientific, Folsom, CA), as described by Theander, Åman, Westerlund, Andersson, and Pettersson (1995). About 90–95% of the monomeric composition was glucose. NMR confirmed the structure to be a linear chain of β-(1 → 3) glucopyranosyl units with β-(1 → 2) branches, as previously described by Dueñas-Chasco et al. (1997).

2.3. Molar mass analysis using asymmetrical flow field-flow fractionation – multiangle light scattering – refractive index

2.3.1. Sample preparation

Sample solutions for asymmetrical flow field-flow fractionation (AFlFFF) were prepared by dissolving the polysaccharide material in distilled water, to a sample mass concentration of 0.10% w/w. The samples were analysed at room temperature (~26 °C) without heating and after heating at 80 °C for 10 min. Sample and carrier solvent for the AFlFFF was 10 mM $NaNO_3$ with 0.002% NaN_3 added to prevent bacterial growth.

2.3.2. Instrumentation

The separation takes part in thin, flat channels, along which a carrier liquid is pumped continuously, generating a flow, which transports the injected sample axially along the channel. Size separation is initiated by a secondary flow called the cross flow, which generates a force (perpendicular to the channel flow) that compels the components to accumulate close to one of the walls called the accumulation wall, which consists of a permeable ultrafiltration membrane (Andersson, Wittgren, & Wahlund, 2001; Wittgren, Wahlund, Andersson, & Arfvidsson, 2002). Diffusion counteracts this movement, resulting in differently sized components differing in their positions above the accumulation wall (Andersson et al., 2001; Wittgren et al., 2002). Differences in diffusion coefficients (differences in size and shape) result in the transportation of various sample components at varying speeds and thus, the sample components will have different retention times. The peak maximum retention time (elution time), t_r, is given by the formula (Wahlund & Giddings, 1987),

$$t_r \approx \frac{w^2 F_c t^0}{6 V^0 D} \tag{1}$$

in which, w is the thickness of the channel, F_c the cross flow-rate, t^0 the void time of the channel, V^0 the geometric volume of the channel, and D the diffusion coefficient of the sample polymer. By combination with the Stokes–Einstein equation, the hydrodynamic radius of the sample polymer can then be derived as (Wittgren, Wahlund, Dérand, & Wesslén, 1996)

$$r_H \approx \frac{kTV^0}{\pi \eta t^0 F_c w^2} \cdot t_r \tag{2}$$

where k is Boltzman's constant, T the absolute temperature, and η the viscosity coefficient. Thus, there is a direct proportionality between the hydrodynamic radius and the retention time. The percentage relative error in the estimated r_H is $\leqslant$10% for retention times $\geqslant$2.3 void times, i.e., in the present work $\geqslant$0.7 min.

The instrument used in this study was an Eclipse F asymmetrical flow FFF instrument connected to a Dawn DSP multiangle light scattering (MALS) detector and an Optilab DSP differential refractive index (DRI) detector, both measuring at 632.8 nm (Wyatt Technology, Santa