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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

Barbara, CA). A pump, with an in-line vacuum degasser and an auto-sampler (1100 series, Agilent Technologies, Palo Alto, CA) delivered the carrier flow and handled the sample injection onto the FFF channel. The channel had a nominal thickness of 350 μm. A filter-holder in Teflon with 20 nm pore size aluminium oxide filter (Anodisc 25 Cat.no. 6809-6002, Whatman International, Maidstone, UK) was placed between the pump and the channel in order to ensure that, the particle-free carrier entered the channel. A polyethylethylketone (PEEK) pre-column filter with a 2 μm PEEK frit (cat.no. A.355 and A.700, Upchurch Scientific, Oak Harbor, WA) was placed between the channel (250 μm) and the MALS detector in order to remove larger impurities, which could otherwise disturb the MALS measurements and clog the RI detector. The ultrafiltration membrane of the accumulation wall was made of regenerated cellulose with a cut-off of 10 kDa (C010F, Nadir filtration, Wuppertal, Germany).

The sample was injected onto the channel at a flow-rate of 0.4 ml min^{-1} for 1.0 min in order to rinse the sample loop. Two sample mass concentrations (50 and 100 μg) were analysed. The focusing flow-rate of 1.0 ml min^{-1} was used. The cross flow-rate (V_c) was started at 1.0 ml min^{-1} and decayed exponentially with a $\frac{1}{2}$-time of 4.0 min. The outlet flow-rate was kept constant at 1.0 ml min^{-1} during the entire separation.

The light scattering data were processed using the Astra software (Wyatt Technology). The molar mass and the root mean square (rms) radius were obtained by the Berry's method (Berry, 1966), fitting a line to the data obtained at 52–121° scattering angle. The lowest scattering angles 14–43°, were not included because they were too imprecise. The term containing the second virial coefficient was assumed to be negligible. No determination of the refractive index increment value (dn/dc) was made. Instead, a value (dn/dc) of 0.145 ml g^{-1} was used based upon the literature. The dn/dc value for most polysaccharides in water is reported to be between 0.13 and 0.15 (Branderup & Immergut, 1989). A 2% error in dn/dc propagates linearly and hence there is an uncertainty of ~10% in the obtained molar mass values due to the uncertainty in the chosen dn/dc value.

2.4. Rheological characterisation

2.4.1. Sample preparation

Solutions for rheological measurements were made in sealed glass vials and all rheological measurements were carried out at 20 °C. Dissolution of the commercial cereal β-glucan material in distilled water was aided by heating (~70 °C) and gentle stirring.

The EPS material was treated in four different ways: (a) sample was dissolved by gentle stirring (300 rpm); (b) sample was treated in a similar manner as in (a) but was also heated at 80 °C for 10 min and then cooled to room temperature; (c) sample was treated in a similar manner as in (b) and then allowed to equilibrate in the cold room overnight; (d) sample was stirred at high speed (1100 rpm) for 5 min.

2.4.2. Instrumentation

The viscoelastic properties of the polymers were studied by subjecting the samples to harmonically varying stress. The experiment was performed on a controlled-strain Bohlin VOR rheometer (Metric Analysis, Stockholm, Sweden), using a torque bar with a rating of 0.25 g cm. The temperature was controlled using a water bath and maintained at 20 ± 0.1 °C. The polysaccharides were squeezed in between stainless steel parallel plate systems (diameter = 15 mm, plate gap = 0.3 mm) and subjected to oscillating rotational deformations with frequencies from 0.01 to 10 Hz, at a strain of 5%. Afterwards a strain sweep from 0.01% to 10% at 2 Hz was performed in order to check that the frequency sweep was run in the linear viscoelastic region. This was to ensure that the amplitude was small enough, so as to avoid any interference with the microstructure of the sample. The data of the rheological measurements were analyzed using supporting rheometer software 900515 (version 2).

Intrinsic viscosity, $[\eta]$, which is the viscosity at infinite dilution (the point at which the effects of the different particles are independent of each other) was also determined. The relative viscosity, η_{rel} was calculated, using water as a reference solvent and a Kraemer plot of $\ln(\eta_{rel})/c$ was plotted. The intrinsic viscosity was calculated by extrapolation to zero-concentration.

2.5. Molecular modeling

SWEET, which is a web-based three-dimensional (3D) construction tool for oligo- and polysaccharides, was used to model 3D conformations of the cereal β-glucan and EPS. SWEET generates reliable 3D models by linking together pre-constructed 3D molecular templates of monosaccharides in a specific manner as determined by the sequence information, followed by molecular dynamics optimization of molecular interactions (Bohne, Lang, & von der Lieth, 1999). Sequence information for representative polysaccharide fragments was provided to the software as follows. For the EPS from Pd, a polysaccharide sequence fragment consisting of 15 back bone residues and seven side chains was generated according to previously published structural information (Dueñas-Chasco et al., 1997). In the case of the cereal β-glucan, a 15 monomer linear sequence was generated with alternating β-(1-3)(1-4) linkages (see Fig. 5 legend). SwissPDB Viewer (Guex & Peitsch, 1997) was used to calculate the hydrogen bonds and to visualize the generated 3D models generated by SWEET.

3. Results and discussion

3.1. Asymmetrical flow FFF-MALS–RI

The combination of asymmetrical flow FFF with MALS and RI provides an efficient method for determining the molar mass, radius of gyration (root mean square radius),

and also their distributions across the size spectrum in the separated fractions of water-soluble polymers. This method is used to separate macromolecules and colloidal material according to their sizes (Giddings, 1993; Wahlund & Giddings, 1987) and it has been used to analyse the molar mass distribution of modified starches (Wittgren et al., 2002).

In this study, the EPS was suspected to have a large polydispersity and therefore a programmed and exponentially decaying cross flow was used in the FFF analysis. In cases of polymers with large polydispersity, a programmed decaying cross flow may result into hastened elution and sharpening of peaks (Wahlund, Winegarner, Caldwell, & Giddings, 1986). As seen from the RI and MALS fractograms in Fig. 1, the sample components were eluted over a broad time range and thus, were very dispersed in size as expected. Two different sample mass concentrations were analysed, 50 and 100 μg. They gave similar results, which demonstrated that no serious overloading had occurred. The duplicates showed that the reproducibility was good. The RI fractograms revealed the presence of two partially resolved population sizes. The first population was eluted between 1 and 2.5 min and the major, second population was eluted between 2.5 and 15 min, indicating that the latter had a relatively larger hydrodynamic size corresponding to a high molar mass. From the RI peak integration, the material eluted before 2.5 min represented about 20% of the total area under the curve. The second population resulted in a much greater response (~70% of the total area under the curve) in the MALS detector than the first population. The first population was unresolved from the 'void' peak occurring at 0.3 min and was hardly visible in the MALS curve. Interestingly, there appeared to be a third size population detected by the MALS, as a shoulder between 15 and 17 min, elution time. This represented an ultra-high molar mass population (~10^6–10^7 g mol^{-1}) and a rms radius range of 140–170 nm. These values were unreliable due to high imprecision and systematic error (erroneous slope of $\log M$ vs. elution time) and could therefore not be used for detailed interpretation. These uncertainties in the first and third populations were caused by difficulties to define baselines, a low MALS signal-to-noise ratio for the first population and a low RI signal-to-noise ratio for the third population.

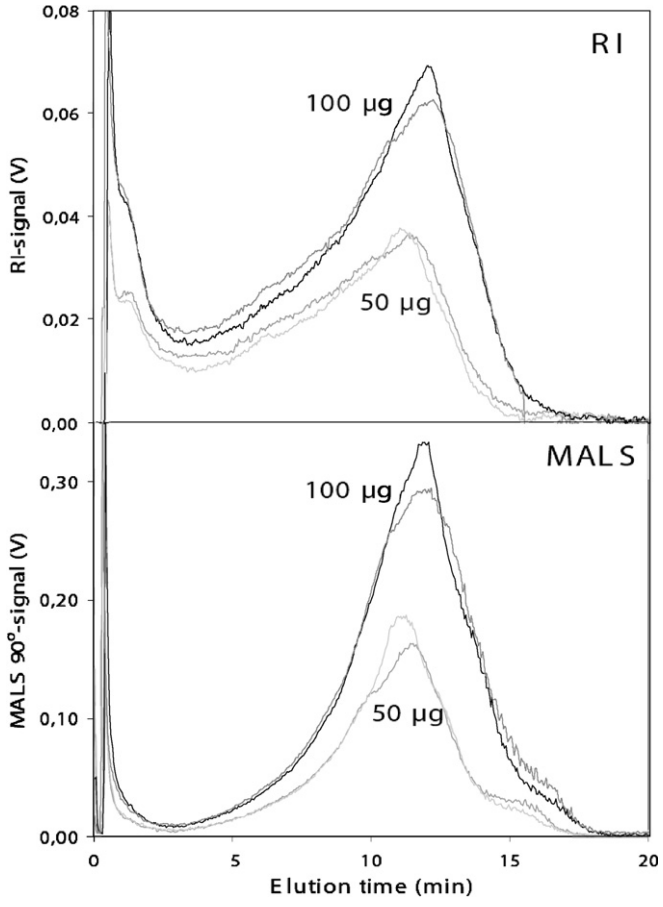

Fig. 1. RI and MALS fractograms (duplicates) of two different sample mass concentrations of EPS (without heating). Outlet flow-rate (V_{out}) = 1.0 ml min^{-1}, cross flow-rate (V_c) = 1.00 ml min^{-1}, exponentially decaying with a $\frac{1}{2}$-time of 4 min. Void time (t^0) ~0.3 min.

In Fig. 2, the obtained molar mass and the rms radius as a function of the elution time are shown for the two sample mass concentrations. They coincided, which again demonstrated the absence of any disturbances from the sample mass overloading.

For the 100 μg sample mass concentration, a weight-average molar mass of 4.0×10^6 g mol^{-1} was obtained for the population between 2.5 and 15 min, representing about 70% of the eluted material (Table 1). The weight-average molar mass obtained for the 50 μg sample mass concentration (3.8×10^6 g mol^{-1}) was not significantly different from that obtained for the 100 μg sample mass concentration.

Lactobacillus lactis ssp. *cremoris* has also been reported to produce EPS with a molar mass value of 1.47×10^6 g mol^{-1} (Tuinier, Zoon, Cohen-Stuart, Fleer, & de Kruif, 1999b). Furthermore, molar mass values of 0.065–3×10^6 and 0.15–2.5×10^6 g mol^{-1} have been observed for oat and barley β-glucans, respectively (Cui, 2001; Cui & Wood, 2000; Gomez, Navarro, Manzanares, Horta, & Carbonell, 1997; Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003; Wood, Weisz, & Mahn, 1991b).

The root mean square (rms) radius is a parameter that is used to describe molecular dimensions and it accounts for the mass distribution around the molecule's centre of gravity. The rms radius was seen to increase with the elution time (Fig. 2) and the average was 100 nm (Table 1). The relationship between the molar mass and the rms radius can be seen in a conformation plot (Fig. 4). Theoretically, for a spherical homogeneous (constant density) polymer, the molar mass should increase with the cube of the polymer rms radius, that is, $r_{rms} \propto M^{1/3}$. For flexible chains, the relationship is $r_{rms} \propto M^{0.5-0.6}$ and the value of the exponent depends on the quality of the solvent. Two conformation plots, based on the results from the two different sample mass concentrations (50 and 100 μg) are shown in Fig. 4. The slope was 0.51, indicating that the EPS is a

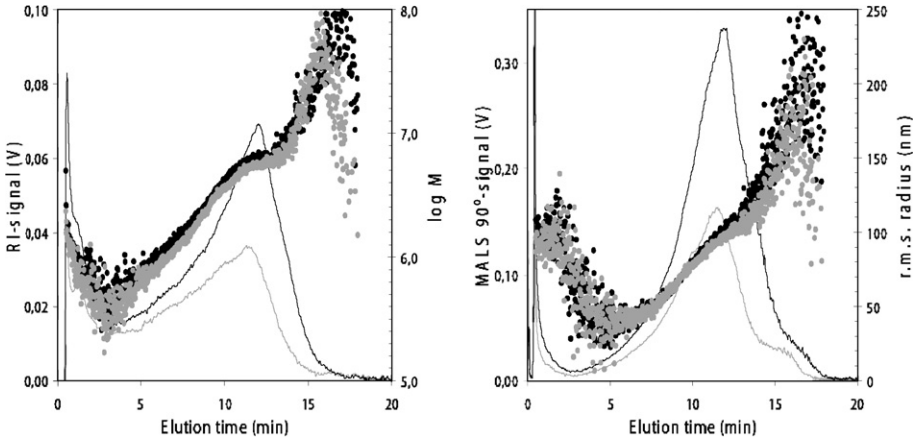

Fig. 2. Logarithm of molar mass and rms radius data overlayed on the RI and MALS fractograms of the two different sample mass concentrations of EPS (50 μg, represented as grey dots and 100 μg, as black dots) with no heat treatment.

Table 1
Weight-average molar mass (M_w), z-average root mean square (rms) radius and the polydispersity index (M_w/M_n) for the EPS analysed at two different sample mass concentrations, with no heat treatment and with heat treatment

Sample	Sample amount (μg)	M_w[a] ($\times 10^{-6}$ g mol^{-1})	rms radius[a] (nm)	M_w/M_n[a]
EPS (no heating)	50	3.8 (7%)	93 (1%)	2.8 (1%)
	100	4.0 (8%)	100 (1%)	2.7 (5%)
EPS (heating at 80 °C for 10 min)	50	3.7 (6%)	99 (6%)	2.3 (8%)
	100	3.5 (1%)	91 (1%)	2.1 (8%)

Values within parentheses are the relative standard deviation between the duplicates ($n = 2$).
[a] Averaged for the second (between 2.5 and 15 min).

flexible chain polymer, according to the classic theory of polymer solutions (Yanagisawa, Shibata, & Isogai, 2005).

Heating the samples at 80 °C for 10 min resulted in similar fractograms (Fig. 3) as compared to those obtained for the unheated samples, except that there was a small shift towards lower elution time. However, no such shift was observed in the molar mass and rms radius distributions, probably because the relative error in the rather complex experimental determination of these parameters is much higher than that in the elution time, such that any change in these parameters was within experimental error and thus not detectable.

Polymers are usually polydispersed, i.e., they occur as a mixture of chains of varying lengths, with different degrees of polymerisation. In order to determine the polydispersity of a polymer, the index M_w/M_n (M_n, number-average and M_w, weight-average) is calculated. In the present study, the polydispersity index was 2.7 (Table 1), confirming the large polydispersity of the EPS. The higher the index, the more disperse is the polymer. An index of 1, means that the polymer is monodisperse (Harding, Vårum, Stokke, & Smidsrød, 1991; Jönsson, Lindman, Holmberg, & Kronberg, 1998). Polymers with an index $\geqslant 2$ are common for industrial grade polymers. Barley β-glucans, with a weight-average molar mass of 0.285×10^6 g mol^{-1}, have shown a polydispersity index of 1.4 and an average rms radius of 35 nm (Clasen & Kulicke, 2003). Moreover, an EPS from *Lactococcus lactis* ssp. *cremoris* NIZO B40 with a molar

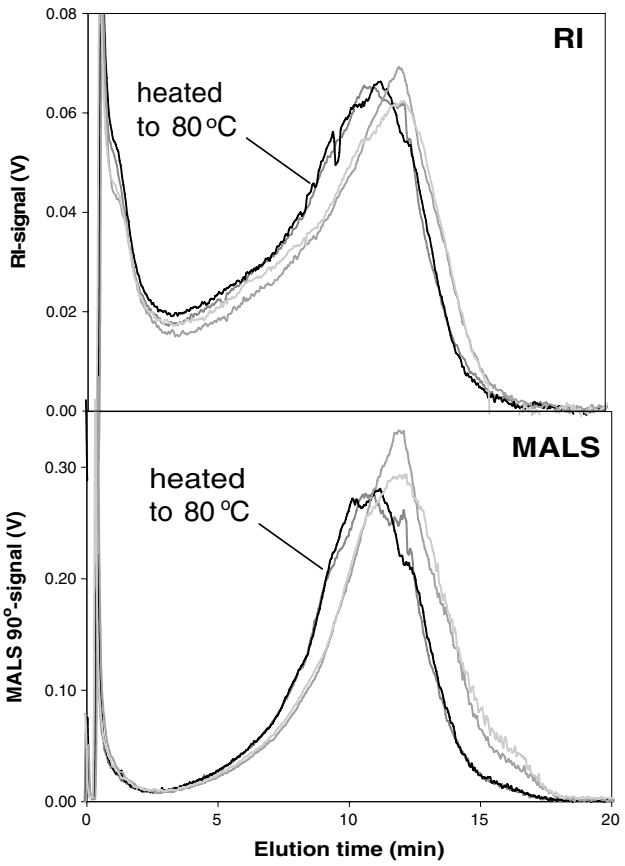

Fig. 3. RI and MALS fractograms (duplicates) of heat-treated EPS (black) overlayed on the fractograms from samples with no heat treatment (grey). Outlet flow-rate (V_{out}) = 1.0 ml min^{-1}, cross flow-rate (V_c) = 1.00 ml min^{-1}, exponentially decaying with a $\frac{1}{2}$-time of 4 min. Void time (t^0) ~0.3 min. Sample mass concentration = 100 μg.

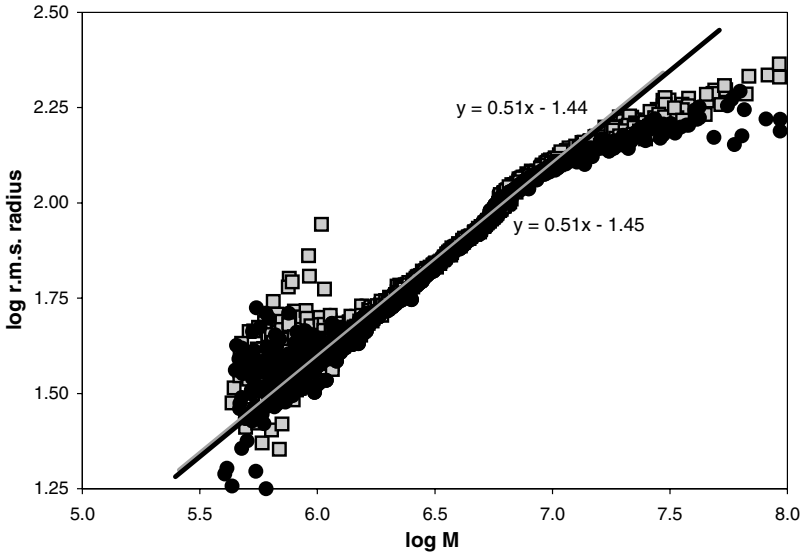

Fig. 4. Conformation plot: logarithm of the rms radius as a function of the logarithm of the molar mass, for the results from the two sample mass concentrations given in Fig. 2. The slopes of the fitted lines are 0.51.

mass of 1.47×10^6 g mol^{-1}, resulted in a polydispersity of 1.13 and an average rms radius of 86 nm (Tuinier et al., 1999a).

3.2. Viscoelastic properties of cereal β-glucan and EPS

Viscoelastic properties of polymers can be determined by dynamic oscillatory analysis, which is the most common method for studying the viscoelastic behaviour of food components (Steffe, 1996). This method has been used to study the behaviour of polymers such as guar gum, pectin and cereal polysaccharides (Böhm & Kulicke, 1999a, 1999b; Lapasin & Pricl, 1995).

The storage modulus, G' and the loss modulus, G'', were measured as functions of frequency (f) for the EPS and cereal β-glucans at 20 °C. Both moduli were seen to increase continuously with the frequency. The variation of G'' and G' moduli with frequency implied that, as the frequency increases, the viscoelastic behaviour changes from being dominated by viscous properties ($G'' > G'$ at lower frequencies) to being dominated by elastic properties ($G' > G''$ at higher frequencies). The frequency sweeps of the two polymers are shown at the concentration of 3.5% in Fig. 5. The above rheological responses are typical of macromolecular dispersions with structural entanglements (Ferry, 1980), and it has been observed for cereal β-glucans in previous studies (Doublier & Wood, 1995; Cui & Wood, 2000; Lazaridou, Biliaderis, & Izydorczyk, 2003).

The G' and G'' curves intersect ($G' = G''$) at a frequency, usually called the cross-over frequency. This frequency marks the transition from liquid-like behaviour to solid-like behaviour. Thus, the polymers can be characterized by the frequency at $G' = G''$, denoted as the cross-over time ($t_{G'=G''} = 2\pi/f_{G'G''}$) of the system and the plateau value of G' at high frequencies ($G'_{\max} = G''(5\,\text{Hz})$). The estimated

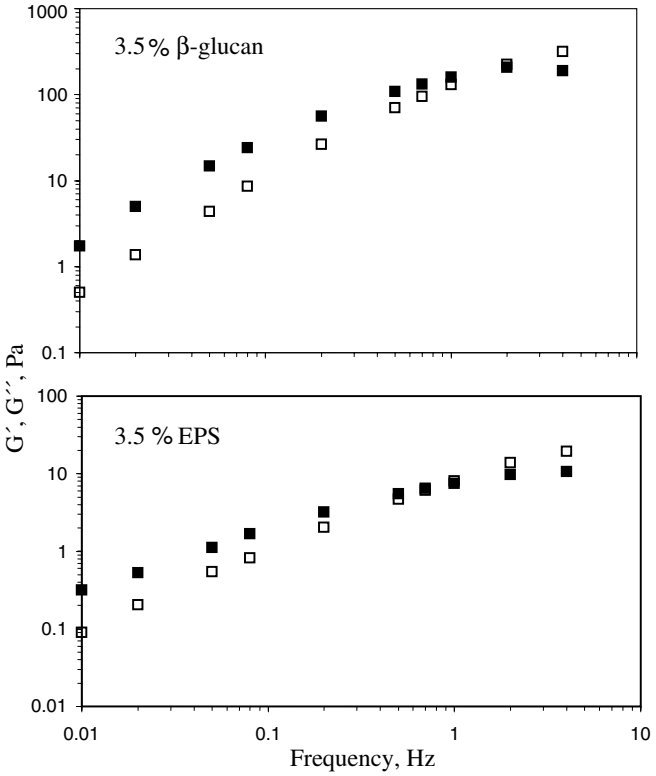

Fig. 5. Frequency sweeps observed with 3.5% w/v cereal β-glucan and EPS. Storage modulus G'(□) and loss modulus G''(■) are plotted as functions of oscillation frequency.

values of $t_{G'=G''}$ and $G'_{\max}$ for the polymers at the various concentrations are shown in Table 2. The $t_{G'=G''}$ and $G'_{\max}$ clearly outlines some of the differences between the polymers. The frequency dependence of G' and G'' at low frequencies is summarized in the power-law constants, n' and n'', which represent the slopes of the linear region of

Table 2
Values of $t_{G'=G''}$, G'_{max}, n' and n'' for cereal β-glucan and EPS

Concentration (%)	G'_{max} (Pa)	$t_{G'=G''}$ (s)	n'	n''
Cereal β-glucan				
2.00	–	–	1.4	1.2
2.50	150.6	0.3	1.4	1.1
2.65	286.4	0.5	1.3	1.0
3.50	330.1	0.6	1.2	1.0
EPS				
3.00	14.0	0.6	1.2	0.8
3.50	24.3	1.1	1.0	0.7
3.75	45.5	2.0	1.0	0.7
4.00	69.7	2.9	0.9	0.7

G' and G'' at low frequencies (G' proportional to $\omega^{n'}$ and G'' proportional to $\omega^{n''}$). For an ideal polymer, following the Maxwell model with one relaxation time, the values of n' and n'' are 2 and 1, respectively.

The frequency sweeps of the two polymers look similar at the 3.5% concentration (Fig. 5), apart from the higher values of G' and G'', over all frequencies for the cereal β-glucan as compared with the EPS. Further, the cross-over point where $G' = G''$, is found at lower frequencies for the cereal β-glucan. At all concentrations investigated, the most obvious difference between the two polymers was seen in G'_{max} for the cereal β-glucan polymer, which was higher than that of the EPS. A greater frequency dependence of G' and G'' in the low frequency region (higher values of n' and n'') was also seen for the cereal β-glucan. It is clear that both polymers deviates from the simple Maxwell model ($n' = 2$) having $1.2 < n' < 1.4$ and $0.9 < n' < 1.2$ for the cereal β-glucan and the EPS, respectively. The frequency dependence of G'' were closer to the Maxwell values ($n'' = 1$) for both polymers (Table 2).

The intrinsic viscosities, $[\eta]$ calculated using the Kraemer method gave values of 6.2 dL/g for the cereal β-glucan and 3.2 dL/g for the EPS. The higher molar mass of the EPS (4.0×10^6 g mol^{-1}) as compared to that of the cereal β-glucan (0.359×10^6 g mol^{-1}) did not seem to favour a higher $[\eta]$ for the EPS. Gomez et al. (1997) reported $[\eta]$ values within the range of 0.73–4.60 dL/g at 25 °C for molar mass values between 0.036 and 0.456×10^6 g mol^{-1}. Izydorczyk, Macri, and Gregor (1998) estimated $[\eta]$ to be between 1.1 and 4.4 dL/g at 20 °C for barley β-glucans, while Böhm and Kulicke (1999a, 1999b) found $[\eta]$ values between 1.15 and 5.40 dL/g at 25 °C for molar mass values in the range of $0.040–0.380 \times 10^6$ g mol^{-1}. For oat β-glucans with similar molar mass values ($0.035–0.250 \times 10^6$ g mol^{-1}), $[\eta]$ estimates within 0.69–3.83 dL/g at 20 °C (Lazaridou et al., 2003) were observed. Furthermore, 2.58–9.63 dL/g at 25 °C were obtained for molar mass values ranging between $0.100–1.200 \times 10^6$ g mol^{-1} (Doublier & Wood, 1995) and 2.0–7.4 dL/g at 20 °C, for molar masses between 0.063 and 0.330×10^6 g mol^{-1} (Vårum & Smidsrød, 1988). It has also been observed in previous studies that lower molar mass β-glucan solutions from barley exhibited higher gelation rates and higher G'_{max} values (Vaikousi, Biliaderis, & Izydorczyk, 2004). Böhm and Kulicke (1999a) reported a similar observation and associated the G'_{max} values with cross-link density of the network structure and thus, with the rigidity of the gel and also made it clear that, G'_{max} predominantly depends on concentration. Gelation properties of the commercial cereal β-glucans and the EPS solutions used in this study were not analysed.

The G'_{max} of the 4% EPS solution, heated at 80 °C for 10 min, was reduced by approximately half of the value obtained for the unheated sample (Fig. 6a). Stirring the EPS solution at high speed (1100 rpm) for 5 min with a magnetic stirrer had no effect on the t_{relax} and G'_{max} values as compared to the gently stirred EPS solutions (Fig. 6b).

It should be noted that the above viscoelastic properties were obtained on pure fractions of the EPS and that this polymer could behave differently in the presence of other components such as cereal β-glucans and proteins. A possible network formation between other polymers and the EPS would most likely affect the viscoelastic properties of the solution. In milk fermented with an EPS-producing strain, EPS strands were observed between the cells and the milk protein network, while a uniform layer of EPS covered the cells (DeVuyst & Degeest, 1999). This could explain why an increase in viscosity was observed for an oat product fermented with an EPS-producing strain (Mårtensson et al., 2000).

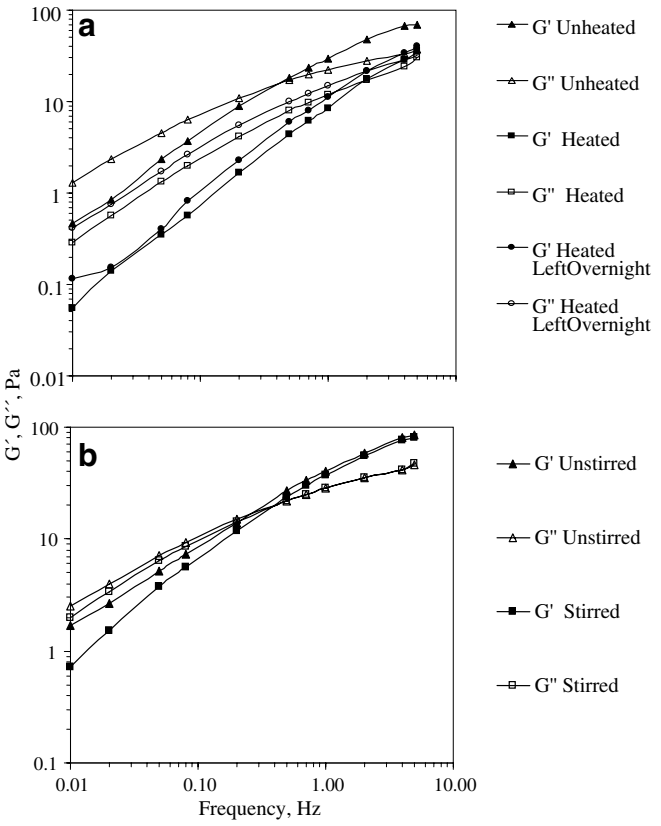

Fig. 6. Frequency sweeps observed with 4% EPS samples analysed at 20 °C; storage modulus G' and loss modulus G'' are plotted as functions of oscillation frequency; (a) heated at 80 °C for 10 min (b) sample was stirred at high speed.

3.3. Three-dimensional molecular modeling

Three-dimensional (3D) modeling of the EPS revealed a molecule with a helical conformation in which the β-(1-3) linked residues constitute the back bone of the helix, and the β-(1-2) linked side-chain branches pack into the helical groove. It should be noted that each side chain consists of one glucose residue (Dueñas-Chasco et al., 1997). Determination of the hydrogen bonding pattern in the molecule revealed an extensive network of intramolecular hydrogen bonding between neighboring side-chain residues, and between side-chain and back-bone glucose residues. Additionally, each back-bone glucose residue was hydrogen bonded to both neighboring residues within the back bone (Fig. 7a). On the other hand, 3D molecular modeling of the cereal β-glucans revealed an extended ribbon-like conformation with fewer intramolecular hydrogen bonds, the only observed hydrogen bonds being at the β-(1-3) linkages (Fig. 7b). Polysaccharides in solution can form 3D networks, in which the chains are cross-linked via interactions between negatively charged polymer chains and positively charged ions, or via interactions of neutral polymer chains through hydrogen bonds and Van der Waals forces (DeVuyst & Degeest, 1999). The involvement of most of the hydrogen bond donors and acceptors within the EPS chain in intramolecular interactions leaves fewer hydrogen bond candidates for potential intermolecular hydrogen bonding. On the other hand, most of the hydrogen bond donors and acceptors within the cereal β-glucans are still available for intermolecular interactions. Such competition between intermolecular and intramolecular hydrogen bonding interactions could explain the lower intrinsic viscosity observed for EPS, despite the higher molar mass. In amphiphilic carboxymethylpullulans, a prevalence of intramolecular interactions over intermolecular interactions, as a result of a high degree of grafting (and molar mass) resulted in lower hydrodynamic radius and intrinsic viscosities (Simon, Dugast, Le Cerf, Picton, & Muller, 2003).

Polymers could exhibit high intrinsic viscosities if long chains of subunits and/or stiff chains are present. Although a relationship between chemical composition of polysaccharides and chain stiffness has not been well established, there are some guidelines (Laws & Marshall, 2001). It has been observed that β-(1-4) linkages in the back bone lead to stiffer chains than do β-(1-2) or β-(1-3) linkages, which lead to more flexible chains. α-Linkages also lead to more flexible chains (Laws & Marshall, 2001). This suggests that for the same concentration of dissolved polysaccharide and given the

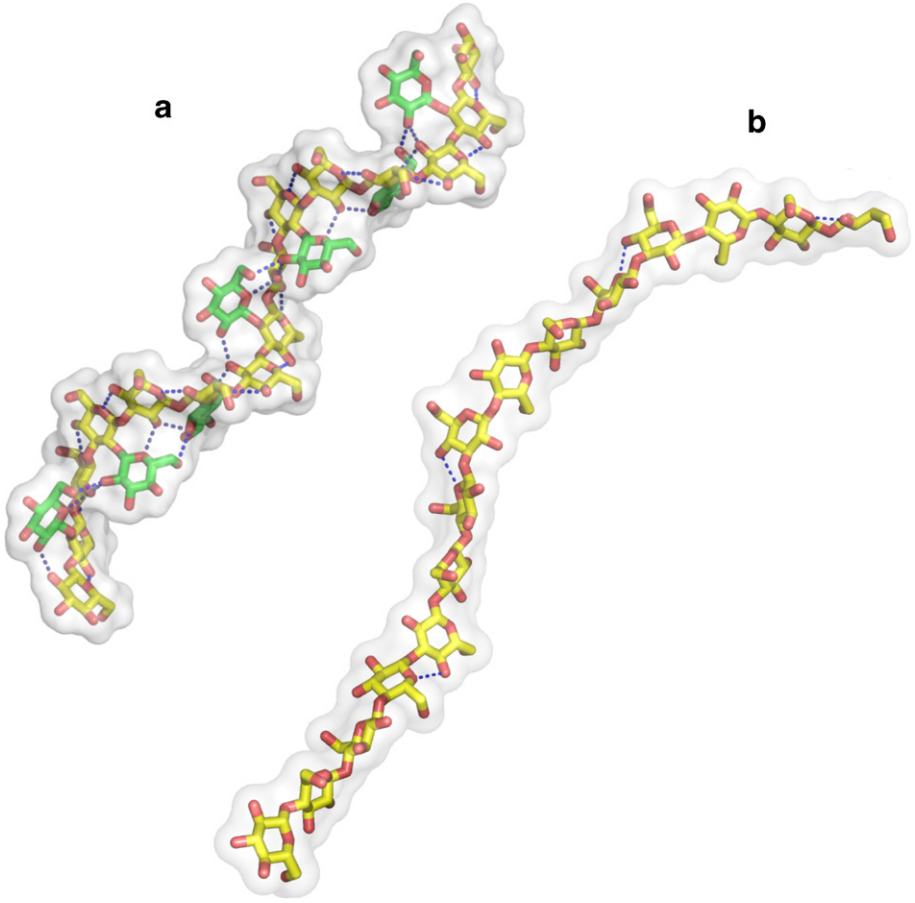

Fig. 7. 3D models of representative fragments of EPS from Pd. (a) and cereal β-glucan (b). The EPS model structure represents the sequence G[=G(−G)=G]$_7$ while the cereal β-glucan model structure represents the sequence [G:G:G=G:G=]$_3$ where (G) is a β-D-glucose residue, (=) is a β-(1-3) linkage, (−) is a β-(1-2) linkage, (:) is a β-(1-4) linkage, and side chains are indicated in parenthesis after the residue they are linked to (each side chain is shown in green and the main chain is shown in yellow).

same molar mass, polysaccharides with a back bone consisting of entirely α-glucose residues or β-(1-2) or β-(1-3) glucose residues will be more flexible than polysaccharides with entirely β-(1-4) glucose residues. It has previously been suggested that the presence of flexible chain EPS in a product may result in a slimy texture or consistency rather than a thickened texture (Laws & Marshall, 2001). As confirmed by the AFlFFF analysis, the EPS from Pd is a flexible chain polymer, due to the presence of entirely β-(1-3) and β-(1-2) linkages. As confirmed by the 3D modeling, the flexibility of the EPS favors the formation of a helical conformation, whereas the prevalence of less flexible β-(1-4) linkages favors an extended conformation in cereal β-glucan. Although the branched nature of the EPS already results in a more compact structure per unit monomer compared to the cereal β-glucans, the helical conformation of the EPS results in further shortening of the chain lengths and an even more compact structure compared with the extended cereal β-glucan. The 3D models generated for the EPS consisted of a back bone of 15 glucose residues and 7 side-chain residues. Therefore, for the same back-bone chain length, the EPS has ~1.5 times more monomers compared to cereal β-glucan. Furthermore, the chain lengths calculated from the EPS and cereal β-glucan models were 5.4 nm (0.25 nm per unit monomer) and 6.6 nm (0.44 nm per unit monomer), respectively. Thus, helicity alone results in about 20% reduction in chain length for the same number of back bone residues. Hence, the cumulative effect of branching and helicity results in about 50% shortening of the chain length per unit monomer of the EPS as compared with the unbranched extended cereal β-glucan. This observation is consistent with the lower intrinsic viscosity observed for the EPS as compared with the cereal β-glucans.

The slightly lower AFlFFF elution time of EPS sample heated at 80 °C for 10 min can be attributed to a decrease in hydrodynamic radius (Eq. (2) and Fig. 3), which could result from either a decrease in molar mass or a conformational change, or both. However, it is unlikely that a decrease in molar mass was responsible for the slightly lower elution time seen for the EPS heated at 80 °C (Fig. 3), since the solvent conditions (deionised water) were not consistent with conditions that could lead to hydrolysis of polysaccharide chains. The most likely explanation, therefore, is that heating at 80 °C for 10 min resulted in an irreversible conformational change in the polymer, resulting into more compact molecules than in the unheated sample. Thus, the heated EPS is likely to involve more non-local intramolecular interactions than the unheated EPS. The reduction in G'_{max} is consistent with a collapse of the polymer chains into more compact aggregates, with fewer networks of intermolecular interactions and a lower hydrodynamic radius. Thermally driven conformational transitions have been observed during molecular dynamic simulations of (1-6)-linked polysaccharides (Lee, Nowak, Jaroniec, Zhang, & Marszalek, 2004). It is possible that similar transitions were induced in the EPS on heating, leading to conformational transitions that favour the formation of more compact structures with lower hydrodynamic radius, and thus lower elution time and lower G'_{max}.

4. Conclusion

Pediococcus damnosus 2.6 produced an exopolysaccharide with a β-glucan structure with a weight-average molar mass of 4×10^6 g mol^{-1}. This was comparable to those of other macromolecules currently used in the food industry. The EPS displayed viscoelastic properties typical of a macromolecular solution with structural entanglements and thus, could be used as a thickener in industrial applications.

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References

Andersson, M., Wittgren, B., & Wahlund, K.-G. (2001). Ultrahigh molar mass components detected in ethylhydroxyethyl cellulose by asymmetrical flow field-flow fractionation coupled to multiangle light scattering. *Analytical Chemistry, 73*, 4852–4861.

Berry, G. C. (1966). Thermodynamic and conformational properties of polystyrene. Light scattering studies on dilute solutions of linear polystyrenes. *Journal of Chemical Physics, 44*, 4550–4564.

Böhm, N., & Kulicke, W.-M. (1999a). Rheological studies of barley (1-3)(1-4)-β-glucan in concentrated solution: investigation of the viscoelastic flow behaviour in the sol-state. *Carbohydrate Research, 315*, 293–301.

Böhm, N., & Kulicke, W. M. (1999b). Rheological studies of barley $(1 \rightarrow 3)(1 \rightarrow 4)$-β-D-glucan in concentrated solution: mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research, 315*, 302–311.

Bohne, A., Lang, E., & von der Lieth, C. W. (1999). SWEET – WWW-based rapid 3D construction of oligo- and polysaccharides. *Bioinformatics, 15*, 767–768.

Branderup, J., & Immergut, E. H. (1989). *Polymer handbook* (3rd ed.). New York: John Wiley & Sons.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic acid bacteria. *FEMS Microbiology Review, 7*, 113–130.

Cerning, J. (1995). Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. *Lait, 75*, 463–472.

Clasen, C., & Kulicke, W.-M. (2003). Rheo-optical studies of barley $(1 \rightarrow 3)$ $(1 \rightarrow 4)$ β-glucan solution: detection of the flow behaviour of aggregates in the sol state. *Journal of Rheology, 47*(2), 321–335.

Cui, W. (2001). *Polysaccharides gums from agricultural products: processing, structure and functionality*. Lancaster, Pennsylvania: Technomic Publishing Company Incorporated.

Cui, W., & Wood, P. J. (2000). Relationships between structural features, molecular weight and rheological properties of cereal β-D-glucan. In K. Nishinari (Ed.), *Hydrocolloids – Part 1* (pp. 159–168). Amsterdam: Elsevier Science B.V.

DeVuyst, L., & Degeest, B. (1999). Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiological Reviews, 23*, 153–177.

Doublier, J. L., & Wood, P. J. (1995). Rheological properties of aqueous solutions $(1 \rightarrow 3)(1 \rightarrow 4)$-β-D-glucan from oats (*Avena Sativa* L.). *Cereal Chemistry, 72*, 335–340.

Dueñas-Chasco, M. T., Rodríguez-Carvajal, M. A., Mateo, P. T., Franco-Rodríguez, G., Esparteo, J. L., Irastorza-Iribas, A., et al. (1997). Structural analysis of the exopolysaccharide produced by *Pediococcus damnosus* 2.6. *Carbohydrate Research, 303*, 453–458.

Ferry, J. D. (1980). *Viscoelastic properties of polymers* (3rd ed.). New York: John Wiley & Sons.

Giddings, J. C. (1993). Colloidal and particulate materials. *Science, 260*, 1456–1465.

Gomez, C., Navarro, A., Manzanares, P., Horta, A., & Carbonell, J. V. (1997). Physical and structural properties of barley $(1 \rightarrow 3)$,$(1 \rightarrow 4)$-β-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. *Carbohydrate Polymers, 32*, 17–22.

Guex, N., & Peitsch, M. C. (1997). Swiss-model and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis, 18*, 2714–2723.

Harding, S. E., Vårum, K. M., Stokke, B. T., & Smidsrød, O. (1991). Molecular weight determination of polysaccharides. *Advanced Carbohydrate Analyses, 1*, 63–144.

Izydorczyk, M. S., Macri, L. J., & Gregor, A. W. (1998). Structure and physicochemical properties of barley non-starch polysaccharides – I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers, 35*, 249–258.

Jönsson, B., Lindman, B., Holmberg, K., & Kronberg, B. (1998). *Surfactants and polymers in aqueous solutions*. Chichester: John Wiley & Sons.

Lambo-Fodje, M. A., Öste, R., & Nyman, M. (2006). Short chain fatty acid formation in the hindgut of rats fed native and fermented oat fibre concentrates. *British Journal of Nutrition*.

Lapasin, R., & Pricl, S. (1995). *Rheology of industrial polysaccharides; theory and applications*. London: Chapman & Hall.

Laws, A. P., & Marshall, V. M. (2001). The relevance of exopolysaccharides to the rheological properties in milk fermented with ropy strains of lactic acid bacteria. *International Dairy Journal, 11*, 709–721.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solutions and gels. *Food Hydrocolloids, 17*, 693–712.

Lee, G., Nowak, W., Jaroniec, J., Zhang, Q., & Marszalek, P. E. (2004). Molecular dynamics simulations of forced conformational transitions in 1,6-linked polysaccharides. *Biophysical Journal, 87*, 1456–1465.

Mårtensson, O., Biörklund, M., Lambo, M. A., Dueñas-Chasco, M., Irastorza, A., Holst, O., et al. (2005). Fermented, ropy, oat-based products reduce cholesterol levels and stimulate the bifidobacteria flora in humans. *Nutrition Research, 25*, 429–442.

Mårtensson, O., Dueñas-Chasco, M., Irastorza, A., Öste, R., & Holst, O. (2003). Comparison of growth characteristics and exopolysaccharide formation of two lactic acid bacteria strains, *Pediococcus damnosus* 2.6 and *Lactobacillus brevis* G-77, in an oat-based, non-dairy medium. *Lebensmittel Wissenschaft und – Technologie, 36*, 353–357.

Mårtensson, O., Staaf, J., Dueñas-Chasco, M., Irastorza, A., Öste, R., & Holst, O. (2000). A fermented ropy non-dairy oat product based on the exopolysaccharide-producing strain, *Pediococcus damnosus* 2.6. *Advances in Food Science, 24*, 4–11.

Paese, M. (2003). Catabolic and anabolic related product formation in lactic acid bacteria. *Licentiate Thesis*, Department of Applied Microbiology, Lund University.

Perry, D. B., Mahon, D. J. M., & Oberg, C. J. (1997). Effects of exopolysaccharide-producing cultures on moisture retention in low fat Mozzarella cheese. *Journal of Dairy Science, 80*, 799–805.

Ricciardi, A., Parente, E., & Clementi, F. (1994). A simple method for the screening of lactic acid bacteria for the production of exopolysaccharide in liquid media. *Biotechnology Techniques, 11*, 271–275.

Ross-Murphy, S. B. (1995). Small deformation rheological behaviour of biopolymer mixtures. In S. E. Harding, S. E. Hill, & J. R. Mitchell (Eds.), *Biopolymer mixtures* (pp. 85–98). Nottingham: Nottingham University Press.

Sebastiani, H., & Zelger, G. (1998). Texture formation by thermophilic lactic acid bacteria. *Milchwissenschaft, 53*, 15–20.

Simon, S., Dugast, J. Y., Le Cerf, D., Picton, L., & Muller, G. (2003). Amphiphilic polysaccharides; evidence for a competition between intra- and intermolecular associations in dilute system. *Polymer, 44*, 7917–7924.

Skendi, A., Biliaderis, C. G., Lazaridou, A., & Izydorczyk, M. S. (2003). Structure and rheological properties of water-soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*. *Journal of Cereal Science, 38*(1), 15–31.

Steffe, F. J. (1996). *Rheological methods in food engineering* (2nd ed.). Michigan: Freeman Press.

Sutherland, I. W. (1977). Bacterial exopolysaccharides, their nature and production. In I. W. Sutherland (Ed.), *Surface carbohydrates of the procaryotic cell* (pp. 27–96). New York: Academic Press.

Theander, O., Åman, P., Westerlund, E., Andersson, R., & Pettersson, D. (1995). Total dietary fibre determination as neutral sugar residues, uronic acids residues, and Klason lignin (the Uppsala method): collaborative study. *Journal of Association of Official Analytical Chemists International, 78*, 1030–1044.

Tuinier, R., Zoon, P., Cohen-Stuart, M. A., Fleer, G. J., & de Kruif, C. G. (1999b). Concentration and shear rate dependence of the viscosity of an exocellular polysaccharide. *Biopolymers, 50*, 641–646.

Tuinier, R., Zoon, P., Olieman, C., Cohen-Stuart, M. A., Fleer, G. J., & de Kruif, C. G. (1999a). Isolation and physical characterisation of an exocellular polysaccharide. *Biopolymers, 49*, 1–9.

Vaikousi, H., Biliaderis, C. G., & Izydorczyk, M. S. (2004). Solution flow behavior and gelling properties of water-soluble barley b-glucans varying in molecular size. *Journal of Cereal Science, 39*, 119–137.

Vandamme, E. J., Bruggerman, G., De-Baets, S., & Vanhooren, P. T. (1996). Useful polymers of microbial origin. *Agro-Food-Industry HI-Technology*, 21–25.

Vårum, K. M., & Smidsrød, O. (1988). Partial chemical and physical characterization of $(1 \rightarrow 3)$,$(1 \rightarrow 4)$-beta-glucans from oat (*Avena Sativa* L) aleurone. *Carbohydrate Polymers, 9*, 103–117.

Wahlund, K.-G., & Giddings, J. C. (1987). Properties of an asymmetrical flow field-flow fractionation having one permeable wall. *Analytical Chemistry, 59*, 1332–1339.

Wahlund, K.-G., Winegarner, H. S., Caldwell, K. D., & Giddings, J. C. (1986). Improved flow field-flow fractionation system applied to water-soluble polymers: programming, outlet stream slitting and flow optimization. *Analytical Chemistry, 58*, 573–578.

Wittgren, B., Wahlund, K.-G., Dérand, H., & Wesslén, B. (1996). Aggregation behavior of an amphiphilic graft copolymer in aqueous medium studied by asymmetrical flow field-flow fractionation. *Macromolecules, 29*, 268–276.

Wittgren, B., Wahlund, K.-G., Andersson, M., & Arfvidsson, C. (2002). Polysaccharide characterisation by flow field-flow fractionation-multiangle light scattering: initial studies of modified starches. *International Journal of Polymer Analytical characterisation, 7*, 19–20.

Wood, P. J., Weisz, J., & Mahn, W. (1991b). Molecular characterisation of cereal β-glucans. II. Size exclusion chromatography for comparison of molecular weight. *Cereal Chemistry, 68*, 530–536.

Yanagisawa, M., Shibata, I., & Isogai, A. (2005). SEC-MALLS analysis of softwood kraft pulp using LiCl/1,3-dimethyl-2-imidazolidinone as an eluent. *Cellulose, 12*, 151–158.