Impact of shear treatment on rheological and molecular properties of microbial exopolysaccharides

Carsten Nachtigall[1], Christiane Berger[1], Tijana Kovanović[1], Daniel Wefers[2], Doris Jaros[1], and Harald Rohm[1]

[1]Chair of Food Engineering, Technische Universität Dresden, 01062 Dresden, Germany
[2]Department of Food Chemistry and Phytochemistry, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany

ABSTRACT

A shear treatment of fermented media during starter culture production can improve the separation of cells from the exopolysaccharide (EPS) containing medium. Apparent viscosity of media with EPS from *Streptococcus thermophilus* was reduced by approx. 25 % when a shear treatment in laboratory scale was performed; this can be linked to a simultaneous decrease in molecular mass. In aqueous solutions of dextran and isolated EPS, high intensity shearing with a microfluidizer led to a pronounced reduction of viscosity, intrinsic viscosity and molecular mass due to a structural disruption of main and side chains of the polymers.

INTRODUCTION

During starter culture production the separation of cells from the fermentation medium is a crucial step, especially in case of exopolysaccharides (EPS) producing lactic acid bacteria. EPS remain either bound to the cells (capsular EPS, cEPS), or are liberated into the medium as free EPS (fEPS). In addition, some strains are able to produce viscosity enhancing fEPS (further denoted as ropy fEPS). Shear treatment of the fermented medium was found to improve separation capability. The aim of this study was to link shear-induced changes of the rheology of the EPS to their respective molecular characteristics.

MATERIALS AND METHODS

Streptococcus thermophilus DGCC7710, known to produce ropy fEPS and cEPS, was cultivated in enriched whey permeate medium (100 g/L) in a 70 L bioreactor (Applikon® Biotechnology, Schiedam, The Nederlands) under anerobic conditions at 40 °C and pH 6.0, kept constant by adding 10 mol/L NaOH. Cells were then removed by crossflow filtration (Sartorius Stedim Biotech GmbH, Göttingen, Germany) using 0.1 µm membrane cassettes. The permeate was further concentrated and dialysed against demineralized water (molecular weight cut-off: 5 kDa) and EPS precipitated by adding 2 volume units of cold acetone. After centrifugation, EPS were resuspended in demineralized water and freeze-dried.

Dextran from *Leuconostoc mesenteroides* was purchased from Sigma-Aldrich Chemie GmbH, Munich, Germany.

Fermented media, cell-free media (obtained by centrifugation) and aqueous solutions of EPS and dextran were subjected to shearing in laboratory scale with a T25 digital Ultra-Turrax®, equipped with the S25 N-10G dispersion tool (IKA-Werke GmbH & Co. KG, Staufen, Germany). Shear speeds adjusted to 11,000, 19,000 or 24,000 rpm for 140 s generated energy inputs of 0.04, 0.21 and 0.43 kJ/mL, respectively. An enhanced shear treatment was achieved using an M-110EH-30 Microfluidizer® (Microfluidics Corporation,

Newton, Massachusetts, USA). Samples circulated successively through a H210Z interaction chamber (200 µm) and different auxiliary processing modules (Table 1) at constant pressure and a heat exchanger for cooling.

Table 1. Shearing conditions for microfluidizer treatment.

APM[a]	Shearing gap [µm]	Time [min]	Pressure [MPa]	Flow rate [mL/min]
Blank	200	30	40	485
H10Z	100	60	100	325
JR20Z	50	60	100	115

[a] Auxiliary processing module.

Dynamic viscosity η of cell-free supernatants was determined with a LOVIS rolling ball viscosimeter (Anton Paar GmbH, Ostfildern, Germany) assuming ρ = 1.00 g/cm³. After equilibration at 20 °C, rolling time (t) was measured six-fold at an angle of 70° using gold-coated steel balls (d = 1.50 mm, ρ = 7.88 g/cm³) and a 1.59 mm capillary. EPS and dextran solutions of different concentration (c_{sample}) were measured and specific viscosity η_{sp} was calculated by

$$\eta_{sp} = \frac{t_{sample}}{t_{solvent}} - 1 \quad (1).$$

Based on the equations of Huggins (Eq. 2) and Kraemer (Eq. 3), intrinsic viscosity [η] can be calculated by extrapolating to a sample concentration of zero from plots of $\eta_{sp} \cdot c_{sample}^{-1}$ or $\ln(\eta_{sp} + 1) \cdot c_{sample}^{-1}$ vs. c_{sample}, respectively:

$$\frac{\eta_{sp}}{c_{sample}} = [\eta] + K_H \cdot [\eta]^2 \cdot c_{sample} \quad (2),$$

$$\frac{\ln(\eta_{sp} + 1)}{c_{sample}} = [\eta] + K_K \cdot [\eta]^2 \cdot c_{sample} \quad (3).$$

K_H and K_K represent equation constants.

Weight average molecular mass (M_w) was determined by size exclusion chromatography (SEC) as described previously[1]. Briefly, samples at a concentration of 1-2 g/L were filtered through a 0.45 µm filter and measured at room temperature with an AZURA Assistant ASM 2.1L GPC system coupled to a Smartline 2300 RI detector (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany), and equipped with three columns (1x PSS Suprema 100 Å + 2x PSS Suprema 3000 Å) and a pre-column (PSS-Polymer-Standard-Service, Mainz, Germany). The eluent consisted of 50 mmol/L $NaNO_3$ and 7.7 mmol/L NaN_3. Calibration was carried out using pullulan standards with a defined molecular mass (342 Da to 2,560 kDa).

NMR spectra were recorded on an Ascend 500 MHz NMR spectrometer (Bruker, Rheinstetten, Germany) equipped with a Prodigy cryoprobe. Samples were dissolved in D_2O and acetone was used as internal reference. Signal assignments were assigned based on two-dimensional experiments (COSY, TOCSY, HSQC, HMBC) and methylation analysis results.

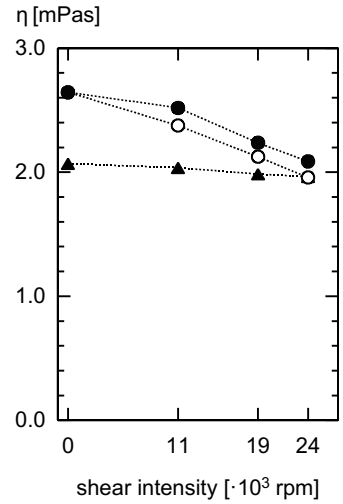

Figure 1. Influence of the shear treatment with the Ultra-Turrax on dynamic viscosity η of the fermented medium (closed circles), cell-free medium (open circles) and isolated fEPS (1 g/L, triangles) of DGCC7710.

RESULTS AND DISCUSSION

Shearing of fermented medium of *S. thermophilus* DGCC7710

The Ultra-Turrax represents an established tool for cell separation in laboratory scale without loss of cell viability[2,3] which is, in turn, essential for starter culture production.

Shear treatment of the fermented media with the Ultra-Turrax led to a decrease in apparent viscosity of 21 % at the maximum shear rate of 24,000 rpm (Fig. 1). Similar results were found when cell-free media were subjected to shear treatment (decrease in viscosity of 26 %). As previously described[1], the decrease in viscosity is most pronounced for ropy EPS and cEPS producing strains. At the same time, SEC measurements revealed that the M_w of the isolated EPS ($2.70 \cdot 10^6$ Da in untreated sample) was reduced by 25 %. That supports the presumption of Jaros et al.[1] who suspected a lowered molecular mass being responsible for viscosity decrease.

Shearing of aqueous dextran and EPS solutions

Shearing aqueous solutions (1 g/L) of isolated fEPS of DGCC7710 with the Ultra-Turrax at the highest shear rate (24,000 rpm) caused only minor changes in the viscosity of the solution (decrease of approx. 5 %, see Fig. 1), and M_w also remained unaffected by the shear treatment. The reason for this phenomenon could not be detected with the used experimental setup.

Dextran, a homopolysaccharide mainly consisting of α-1,6- and α-1,4-linked glucose units and a M_w of $2.25 \cdot 10^5$ Da was used as model EPS. As for EPS isolated from DGCC7710, shearing of dextran solutions (1 g/L) did not alter its molecular mass, so that η remained unaffected. Nearly unchanged [η] for both polymers suggest that these shearing conditions induce no structural changes in polymers (Fig. 2). For fEPS of DGCC7710, [η] was reported to be 0.248 mL/mg.[4] The higher [η] in this study could be the consequence of a differing isolation procedure and therefore different purity of the isolated EPS. Generally, higher

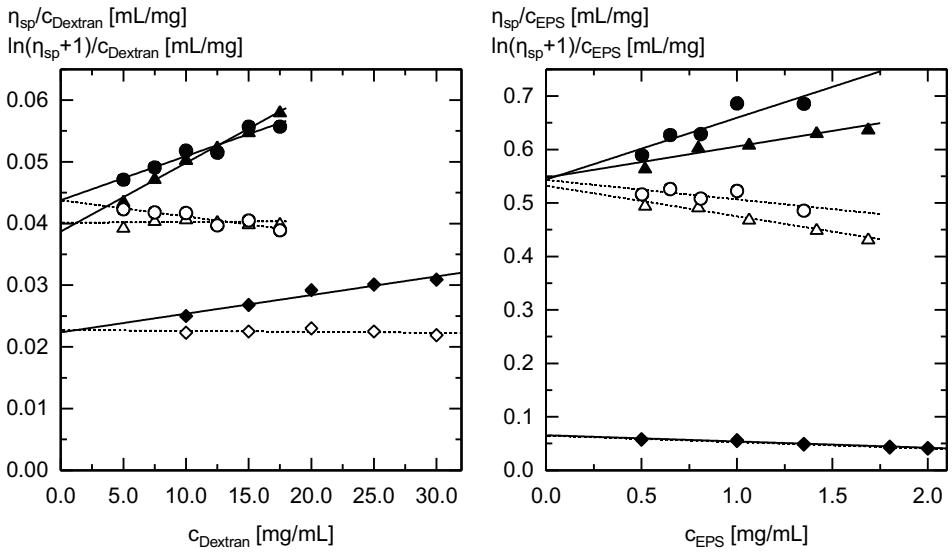

Figure 2. Intrinsic viscosity plot of Dextran (left) and isolated fEPS of DGCC7710 before (circles) and after shearing with Ultra-Turrax (24 000 rpm, triangles) and microfluidizer (50 µm shearing gap, rhombuses). Closed symbols: Huggins extrapolation, open symbols: Kraemer extrapolation.

[η] were found for ropy EPS. As dextran does not enhance viscosity in a comparable amount, [η] is much lower (0.044 mL/mg). Mende et al.[5] reported a similar value of 0.039 mL/mg.

However, the high energy input during shear treatment of cell suspensions with a microfluidizer goes along with cell wall disruption and cell lysis[6], but is also known to improve solubility of carbohydrate polymers[7] and to reduce their molecular mass and viscosity[8]. To regulate the shear intensity, different shearing gaps were used. As can be seen in Fig. 3, shearing results in a pronounced decrease in η and M_w for both dextran and fEPS of DGCC7710. [η] was also lowered dramatically to 0.022 mL/mg and 0.066 mL/mg, respectively.

fEPS of DGCC7710 were characterised as heteropolysaccharides, containing α-1,3-glucopyranose, β-1,3-galactofuranose, β-1,3,6-glucopyranose and β-t-galactopyranose in accordance with Pachekrepapol et al.[9] (Fig. 4).

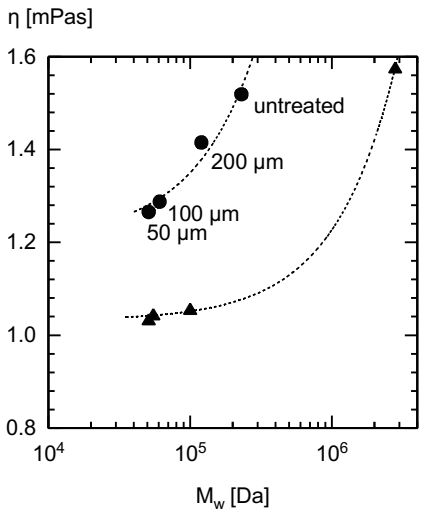

Figure 3. Influence of the shear treatment with a microfluidizer on weight average molecular mass M_w and dynamic viscosity η of aqueous solutions of Dextran (10 g/L, circles) and fEPS of DGCC7710 (0.1 g/L, triangles). Shearing gap is indicated for dextran.

Proton spectra of isolated EPS contained four distinct signals in the anomeric region, which represent the anomeric protons of the four structural units. As can be seen from Fig. 4, shearing did not result in significant changes of signal intensities, which was also confirmed by the signal integrals. These results suggest that the structural composition, neither side chains nor backbone structure, is altered by shearing with microfluidizer.

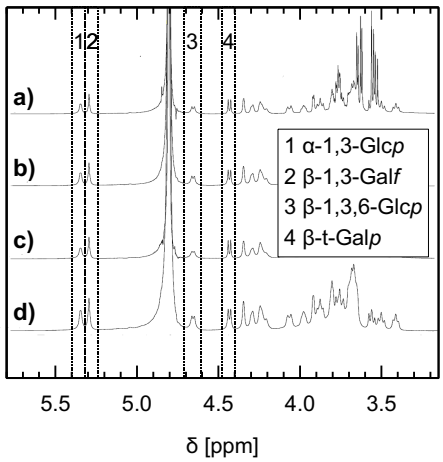

Figure 4. 1H NMR spectra of isolated fEPS of DGCC7710. a)-c) Shear treatment with microfluidizer and 200 µm, 100 µm and 50 µm shearing gap, d) without shear treatment, δ… chemical shift.

The different response to shear treatment can be linked to structural properties via the Mark-Houwink equation:

$$[\eta] = K \cdot M_w^{\alpha} \qquad (4).$$

α indicates the shape of the analysed polymer (hard sphere – fixed rod)[10]. In ongoing experiments, different M_w of isolated EPS are adjusted by a defined shear energy input. Together with the determination of [η], α could explain the different behavior of various types of EPS (ropy and non-ropy fEPS, cEPS).

The shear treatment of EPS also affects the properties of model products. Gelation experiments of chemically acidified milk

were performed in a thromboelastometer. First experiments showed that, compared to untreated EPS, shear induced changes in EPS affected gelation behavior differently when added prior to acidification.

ACKNOWLEDGMENTS

Financial support was received from Deutsche Forschungsgemeinschaft (Bonn, Germany) under the grant number JA 2033/1-2.

Thanks are due to the group of Prof. S. Fischer, Institute of Plant and Wood Chemistry, Technische Universität Dresden, for the performed shearing experiments with the microfluidizer.

REFERENCES

1. Jaros, D., Mende, S., Häffele, F., Nachtigall, C., Nirschl, H., Rohm, H. (2018). "Shear treatment of starter culture medium improves separation behavior of *Streptococcus thermophilus* cells", *Eng. Life Sci.*, **18**, 62–69.

2. Champagne, C.P., Raymond, Y., Tompkins, T.A. (2010). "The determination of viable counts in probiotic cultures microencapsulated by spray-coating", *Food Microbiol.*, **27**, 1104–1111.

3. Champagne, C.P., Ross, R.P., Saarela, M., Hansen, K.F., Charalampopoulos, D. (2011). "Recommendations for the viability assessment of probiotics as concentrated cultures and in food matrices", *International J. Food Microbiol.*, **149**, 185–193.

4. Mende, S., Mentner, C., Thomas, S., Rohm, H., Jaros, D. (2012). "Exopolysaccharide production by three different strains of *Streptococcus thermophilus* and its effect on physical properties of acidified milk", *Eng. Life Sci.*, **12**, 466–474.

5. Mende, S., Peter, M., Bartels, K., Dong, T., Rohm, H., Jaros, D. (2013). "Concentration dependent effects of dextran on the physical properties of acid milk gels", *Carbohydr. Polym.*., **98**, 1389–1396.

6. Stupak, R., Makauskas, N., Radzevičius, K., Valančius, Z. (2014). "Optimization of Intracellular Product Release from *Neisseria denitrificans* Using Microfluidizer", *Prep. Biochem. Biotech.*, **45**, 667–683.

7. Guraya, H., Lima, I., Champagne, E. (2010). "Method of creating starch-like ultra-fine rice flour and effect of spray drying on formation of free fatty acid", *Starch*, **62**, 108–116.

8. Lagoueyte, N., Paquin, P. (1998). "Effects of microfluidization on the functional properties of xanthan gum", *Food Hydrocoll.*, **12**, 365–371.

9. Pachekrepapol, U., Lucey, J.A., Gong, Y., Naran, R., Azadi, P. (2017). "Characterization of the chemical structures and physical properties of exopolysaccharides produced by various *Streptococcus thermophilus* strains", *J. Dairy Sci.*, **100**, 3424–3435.

10. Villay, A., Lakkis de Filippis, F., Picton, L., Le Cerf, D., Vial, C., Michaud, P. (2012). "Comparison of polysaccharide degradations by dynamic high-pressure homogenization", *Food Hydrocoll.*, **27**, 278–286.