Relating particular properties of *Lactococcus lactis* exopolysaccharides to the rheological characteristics of fresh and white cheese

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ABSTRACT

Techno-functionality of *in situ* exopolysaccharides (EPS) of 2 different *Lactococcus lactis* (LL) strains affected the texture of fresh cheese and feta-style white cheese. For ropy free and cell-bound EPS of LL-1 higher yield stress and lower particle size for fresh cheese, and a softer and more elastic texture for white cheese were found compared to non-ropy free EPS of LL-2. The rheological properties of fresh and white cheese of LL-1 were mainly affected by higher molecular mass of their EPS than for LL-2.

INTRODUCTION

For the manufacture of fermented dairy products (e.g. yoghurt, cheese) lactic acid bacteria (LAB) are used. Some strains are capable of synthesising exopolysaccharides (EPS) during fermentation, which induce texture-enhancing effects[1] and are therefore increasingly used in the dairy industry. EPS can be distinguished by their location into free EPS (fEPS) present in the medium and cell-bound EPS (cEPS), attached to the bacterial cells, and by the effect they evoke into ropy and non-ropy EPS[2].

The texture of the final products depends on the strain specific structure and macromolecular properties of the EPS (e.g. intrinsic viscosity, molecular mass). Whether their high water-binding capacity or interactions between proteins and EPS are mainly responsible for texture effects, need to be clarified. This study attempts to relate physical properties of *in situ* produced EPS to the rheology of fresh and feta-style white cheeses.

MATERIAL AND METHODS

Cultures

Two *Lactococcus lactic* (LL) strains (LL-1 produces ropy fEPS and cEPS, LL-2 non-ropy fEPS) were used as direct starters for the inoculation of milk. Direct starters were obtained by fermentation of whey permeate medium (12 g/100 g dry matter with 1 g/100 g tryptone) after inoculation with 10 mL/L preculture at 32 °C to pH 4.7, centrifugation of 30 mL for fresh cheese and 70 mL for white cheese production at 19000 g (15 min, 20 °C) and decantation of the supernatant. The cell pellets were stored at -80 °C.

Fresh and white cheese production in laboratory scale

Reconstituted skim milk (dry matter 14.5 g/100 g) was heated to 90 °C for 10 min, subsequently cooled to 30 °C and inoculated with direct starters of LL-1 or LL-2. Fermentation was stopped at pH 4.6 by cooling to 20 °C. Milk gels were further processed by shearing, heating (50 °C, 5 min) and centrifugation at 40 °C[3]. Centrifugal force was adjusted to obtain 40 g fresh cheese from 100 g milk gel after whey separation[4].

Pasteurised milk (15 g/kg fat) purchased from a local supermarket was inoculated with direct starters at 33 °C and 2 g/L $CaCl_2$ was added. After 3 h pH was adjusted with lactic acid (50 mL/L) to pH 6.4 and chymosin was added at 30 IMCU/L for 25 min. Cutting (wire distance = 28 mm) and stirring time were adjusted to obtain cheese with comparable dry matter of 290 g/kg. For whey separation cheese curd was filled into perforated moulds (d = 100 mm) and stored for 18 h at 21 °C. White cheese was ripened in brine with 50 g/L $NaCl_2$ for 3 weeks.

Physical and rheological characterisation

Fresh cheese was examined after 1 d of storage at 4 °C. Stress sweeps were recorded using parallel plate geometry (d = 40 mm, gap: 1 mm) of an AR-G2 rheometer (TA Instruments, New Castle, USA) at 6.28 rad/s and 15 °C. Storage modulus G' and loss modulus G'' were determined as a function of shear stress τ, which was increased from 0.01 to 1000 Pa with 10 points per decade and 20 s per point (n = 3). Oscillatory yield stress τ_{Osc} was defined as τ at 95 % of G' in the linear-viscoelastic region.

Cylinders (r_0 = 12 mm) were cut from white cheese (stored at 4 °C for 5 d) and deformed by parallel plate geometry at 1 mm/s in a universal testing machine (TA.XTplus, Stable Micro Systems, Godalming, UK) to 50 % of initial height h_0 at 15 °C (n = 5). Force F_t and height h_t were used for the calculation of compression stress σ_t (Eq. 1) and Hencky strain ε_t (Eq. 2)[5]:

$$\sigma_t = \frac{F_t}{{r_0}^2 \pi} \cdot \frac{h_t}{h_0} \quad (1),$$

$$\varepsilon_t = \left| \ln \frac{h_t}{h_0} \right| \quad (2).$$

Particle size distribution of suspended fresh cheese was determined by laser diffraction (Helos KR with Sucell, λ = 633 nm, Sympatec GmbH, Clausthal-Zellerfeld, Germany) and an optical density of 10 - 15 % (n = 3). d_{v90} was calculated as particle size indicator after 5 min equilibration under stirring in a range of 0.5 - 875 µm.

Microstructure of fresh cheese was visualised after staining with Nile blue in a confocal laser imaging microscope (TCS SP5 MP invers, Leica Microsystems GmbH, Wetzlar, Germany) equipped with 63x objective, hybrid detector and laser (λ = 633 nm).

EPS production and isolation

For enhanced EPS production batch fermentation was carried out at 30 °C under anaerobic conditions and an agitator speed of 200 rpm in a 5 L bioreactor (Applikon Biotechnology B.V., Schiedam, the Netherlands). Whey permeate medium (100 g/L) enriched with 2 g/L ammonium sulphate, 10 g/L tryptone, 34 g/L lactose and 10 g/L glucose was inoculated with 10 mL/L preculture. During fermentation, pH of the medium was kept at 6.0 by automatic addition of 10 M NaOH[6]. Fermentation was stopped at the end of exponential growth.

To isolate the fEPS fractions for chemical characterisation, the fermented medium was diluted with buffer (9 g/L NaCl and 2 g/L NaN_3) in equal ratio. Cells were separated at 40 °C by cross-flow microfiltration with a 0.1 µm membrane (Sartorius Sedim Biotech GmbH, Göttingen, Germany) and permeate was further concentrated and dia-filtrated with a 5 kDa membrane. The fEPS fraction in the retentate was precipitated by 2 volume units of acetone, separated by centrifugation (19000 g, 15 min, 4 °C) and freeze-dried.

Size exclusion chromatography

Molecular mass was determined by size exclusion chromatography (AZURA Assistant ASM 2.1L, Knauer Wissenschaftliche Geräte GmbH, Berlin,

Germany) using 3 columns for separation as described previously[7]. EPS fractions were injected at 1.5 mg/mL and molecular mass was calculated by calibration with Pullulan standards.

RESULTS AND DISCUSSIONS

An elastic behaviour (G' > G") was detected for fresh cheese produced with LL-1 and LL-2 in the linear-viscoelastic region ($\tau < 100$ Pa, see Fig. 1). With increasing τ a drop in G' and G" was detected, which was defined as oscillatory yield stress τ_{Osc}. Relative firmness in the linear-viscoelastic region and τ_{Osc} were significant higher for fresh cheese produced with the ropy fEPS and cEPS producing strain LL-1 (G' = 11 ± 4 kPa and τ_{Osc} = 137 ± 67 Pa) compared to LL-2 (G' = 6 ± 1 kPa, τ_{Osc} = 77 ± 30 Pa).

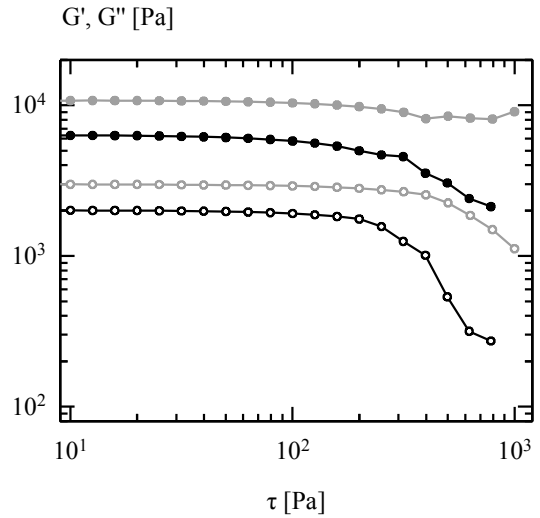

Figure 1. Stress sweep of fresh cheese produced with LL-1 (grey) and LL-2 (black). Storage modulus G' (filled) and loss modulus G" (open).

Fresh cheese with LL-1 revealed lower particle size by laser diffraction ($d_{V,90}$ = 122 ± 20 µm) in comparison to LL-2 (191 ± 22 µm). This was confirmed by confocal laser scanning microscopy, where the aggregates seemed to be less densely packed (see Fig. 2).

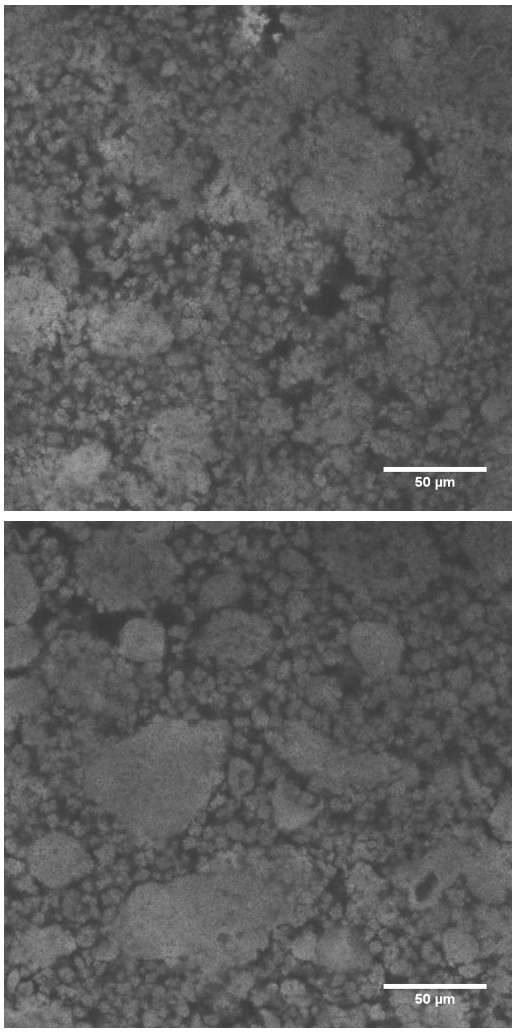

Figure 2. Microstructure of fresh cheese produced with LL-1 (top) and LL-2 (bottom). Protein was stained with Nile blue and visualised by confocal laser scanning microscopy; scale bar = 50 µm.

Fresh cheese is basically a colloidal microgel suspension of casein micelles and whey as continuous phase[8]. Hahn et al.[4] explained a higher relative firmness of fresh cheese with EPS-producers with interactions between EPS and microgel particles, whereas Zhang et al.[9] postulated that interactions are reduced by EPS. A lower yield stress correlated with sensory spreadability[10], which indicates that the non-ropy fEPS of LL-2 resulted in products that are easier to spread than the ropy fEPS and cEPS of LL-1.

With the same 2 strains feta-style white cheese was produced in laboratory scale. Compression curves of white cheese obtained by converting force and height into stress and strain showed an increase until fracture (see Fig. 3).

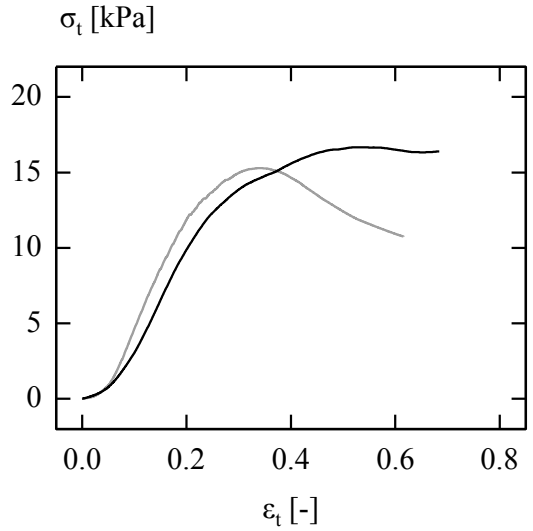

Figure 3. Compression stress σ_t plotted against Hencky strain ε_t of white cheese produced with LL-1 (grey) and LL-2 (black).

The shape of the curves indicates visco-elastic behaviour[5]. White cheese produced with LL-1 showed lower stress at apparent fracture σ_f of 15.4 ± 3.9 kPa compared to the non-ropy fEPS of LL-2 (19.4 ± 3.6 kPa). The corresponding Hencky strain ε_f, representing the deformation at apparent fracture, was lower for LL-1 (0.4 ± 0.1) than for LL-2 (0.6 ± 0.0). σ_f is a measure for sensory firmness and ε_f for sensory elasticity[5]. Lower σ_f and ε_f for white cheese with ropy fEPS and cEPS showed a softer and more elastic texture compared to non-ropy fEPS. Mainly the cEPS are known to have high water-binding capacity and therefore reduce the firmness of white cheese[11].

For the determination of physiochemical properties of EPS and their effect on cheese texture the strains were cultivated in a bioreactor and fEPS fractions were isolated. For the fEPS fraction of LL-1 a higher molecular mass of $3 \cdot 10^6$ Da was found than for LL-2 ($3 \cdot 10^5$ Da), which correlates well with the enhanced effects. These first results indicate that mainly physical properties of the EPS affect their techno-functionality in the final cheese products. The ropy fEPS and cEPS resulted in fresh cheese with higher yield stress and lower particle size, whereas white cheese appeared more elastic and less firm compared to the non-ropy fEPS. Further characterisations (e.g. intrinsic viscosity) are under progress.

ACKNOWLEDGEMENTS

The IGF Projects 18318 BR and 19663 BG of the FEI are supported via AiF within the programme for promoting the Industrial Collective Research (IGF) of the German Ministry of Economic Affairs and Energy (BMWi), based on a resolution of the German Parliament.

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