Dextran modulates physical properties of rennet-induced milk gels

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Summary Physical properties of rennet-induced milk gels as core intermediates of cheese production are mainly affected by milk composition, type and amount of coagulation enzyme and starter culture activity. We investigated model systems of reconstituted milk and dextran, which triggers effects on milk gels similar to exopolysaccharides from lactic acid bacteria. Furthermore, clotting activity (0.02 or 0.04 IMCU mL⁻¹) and milk pH adjusted prior to renneting (6.5–5.7) were studied. A lower pH at renneting resulted in an earlier gelation onset, a higher gelation velocity and gel stiffness. The addition of dextran stabilised the gels especially at higher pH, and microstructural analysis revealed larger, more interconnected protein aggregates. However, at pH 5.7, a reverse effect was observed, indicating a destabilisation of the casein network. The current study indicates that altering milk pH and addition of polysaccharides gives the potential to change textural properties of cheese by affecting rennet-induced gelation.

Keywords Dextran, hydrocolloids, microstructure, milk gel, rennet, rheology.

Introduction Acid- and rennet-induced gel formation are fundamental processes in the production of a large number of milk products. Both are based on the destabilisation of casein micelles, and their subsequent aggregation to a stable protein network that is further processed to a different extent. In case of yoghurt or related products, microbial acidification triggers a pH-dependent release of calcium from the micelles with subsequent structural rearrangements (Lucey, 2008). Production of the majority of cheeses is based on enzymatic milk coagulation by chymosin or similar rennet-like enzymes, supported by mild acidification, either simultaneous or preceding the action of rennet. For some cheeses (for instance, fresh cheese or quarg), acidification is more intense and only supported by milk composition and only supported by a small amount of rennet to increase coagulum firmness and to minimise casein losses. Rennet cleaves the caseinomacropeptide from κ-casein which is located at the casein micelle surface (Fox et al., 2017). Its release results in a decrease of the ζ-potential and thus, in reduced electrostatic repulsion between the remaining para-κ-casein micelles, which aggregate in the presence of Ca²⁺ after approx. 85% of κ-casein is hydrolysed, forming a three-dimensional protein network. The velocity of the enzymatic reaction depends largely on environmental conditions, mainly temperature and pH (Jaros & Rohm, 2017). The rearrangements and interactions which take place during gelation have been summarised by, for example, Horne (2003) and Le Feunteun & Mariette (2008).

Cheese texture is affected by a large number of factors including milk composition, coagulation enzyme, activity of starter or adjunct cultures (acidification, flavour, exopolysaccharide formation) and conditions during whey separation and maturation. The impact of pH and temperature on the kinetics of rennet-induced gel formation was mainly studied using model systems (Zoon et al., 1989; Daviau et al., 2000; Esteves et al., 2003; Jaros et al., 2008), but significant effects were also observed in cheese-making experiments. Ong et al. (2012) produced Cheddar from pre-acidified milk (pH 6.7–6.1) and reported that a higher acidity prior to renneting improved cheese yield and texture. Similar results were obtained for French soft cheese (Verdier-Metz et al., 2001) and Grana Padano (Pretto et al., 2013).

A further approach to enhance cheese yield and texture is through polysaccharides, irrespective whether these are exopolysaccharides (EPS) produced in situ by lactic acid bacteria, or come as additive from microbial (xanthan, dextran), algae (carrageenan) or plant...
sources (guar gum, pectin). Several studies emphasized the positive effects of in situ produced EPS on low fat Cheddar cheese (Rynne et al., 2007; Costa et al., 2010), Mozzarella (Broadbent et al., 2001; Zisu & Shah, 2005) or soft cheese (Hahn et al., 2014), and others deal with regional cheese varieties such as Mexican Manchego (Lluis-Arroyo et al., 2014), Brazilian Prato (Nepomuceno et al., 2016), or Italian Caciotta (Di Cagno et al., 2014). The general outcome is that EPS increase moisture retention and hence cheese yield and that texture and melting properties of fat-reduced cheese are improved. Most studies focus on mature cheeses, but some do also analyse the impact of EPS on coagulation and curd formation (Hassan & Frank, 1997; Rynne et al., 2007). Here, the effects of the in situ EPS are usually explained by their water binding capacity and interactions with milk proteins. The effects of polysaccharides from different sources on product texture strongly depend on type and concentration (Oliveira et al., 2011; Benjamin et al., 2018; Dai et al., 2018; Rubel et al., 2019), and some studies also reported a lower sensory acceptance of low fat cheeses with polysaccharides (Korish & Abd Elhamid, 2012; Cooke et al., 2013). To understand the impact of such additives on microstructure and rheology of rennet gels Tan et al. (2007) and Galante et al. (2017) examined, among others, the impact of guar gum, an uncharged polysaccharide. When present at low concentration (≤0.75 g kg⁻¹), coagulation time was reduced and a continuous, compact protein network was formed. This, however, changed to a disconnected system with low gel stiffness and high syneresis at higher polysaccharide concentration.

For in-depth understanding of the impact of polysaccharides on dairy products, either produced in situ or used as additive, it is useful to investigate model systems. The aim of our study was to analyse the effects of different concentrations of a well-known uncharged polysaccharide, namely dextran with a defined molecular mass, on rennet-induced gelation. Dextran is the only polysaccharide produced by lactic acid bacteria that is approved for being used in the food industry (European Commission, 2001), and it is usually applied as texturizing, stabilising or emulsifying agent in, for instance, bakery and confectionary products and ice cream (Kothari et al., 2015). It has already been shown that dextran affects physical properties of acid milk gels, and we successfully applied it as a model for EPS from Streptococcus thermophilus (Mende et al., 2013). Purpose of the present study was to analyse combined effects of dextran concentration and milk pH during rennet-induced gelation. Therefore, gel formation was studied using (i) milk with pH adjusted to 6.5–5.7 and (ii) milk at continuously decreasing pH induced by addition of a chemical acidulant.

Materials and methods

Materials

Base milk was prepared from low-heat skim milk powder (SMP), dry matter: 968 g kg⁻¹ (Käserei Champignon Hofmeister GmbH & Co. KG, Lauben/Allgäu, Germany) and demineralised water. Calf rennet, certified activity: 101 IMCU mL⁻¹, was from Chr. Hansen A/S (Hørsholm, Denmark), and lactic acid and dextran, specified molecular mass: 5 × 10⁵ Da, were from Merck KGaA (Darmstadt, Germany).

Base milk preparation and renneting procedures

Skim milk powder was dissolved in demineralised water to achieve reconstituted milk with 150 g kg⁻¹ dry matter. After adding 0.3 g kg⁻¹ sodium azide, the milk was stirred at room temperature for 30 min and stored in a cold room overnight. Reconstituted milk was then mixed either with a dextran stock solution (150 g kg⁻¹), diluted stock solutions or water in a ratio of 4:1. The final concentration in the milk was therefore 120 g kg⁻¹ milk solids and zero (reference) or 1, 2.5, 7.5, 12.5 or 30 g kg⁻¹ dextran (cDex).

Prior to renneting, lactic acid was used to adjust milk pH to 6.5, 6.2, 5.9 or 5.7. At a temperature of 34 °C, gelation was induced by adding diluted rennet to achieve a clotting activity of 0.02 or 0.04 IMCU mL⁻¹. For a subset of samples, rennet action was superimposed by a continuously decreasing pH. For this purpose, glucono-δ-lactone (GDL) was added simultaneously in amounts of 3.0 or 4.5 g L⁻¹, resulting in a pH drop from 6.5 to 5.9 or 6.5 to 5.7 within 2 h, respectively.

Physical analysis of milk gels

For analysing gelation kinetics and gel properties, an ARES RFS3 rheometer (TA Instruments GmbH, Eschborn, Germany) was used. Gelation was monitored in duplicate using independently prepared base milk with a Couette device (d_i = 32 mm, d_o = 34 mm, h = 33.5 mm) at 34 °C. After sample loading, the storage modulus G' (Pa) was recorded as a function of time. Strain amplitude γ was 0.003, and angular frequency ω was 1 rad s⁻¹ (Jacob et al., 2011a). After 2 h, frequency sweeps (0.1 rad s⁻¹ < ω < 100 rad s⁻¹, γ = 0.003) were performed.

Forced syneresis S (%) of set milk gel was determined in sixfold (triplicate analysis of independently prepared base milk). Ten millilitre renneted milk was incubated in 15 mL Falcon tubes for 2 h at 34 °C, and cooled 1 h at 6 °C in a water bath. After centrifugation (6 °C, 1000 × g, 20 min; Heraeus BioFuge Stratos, Thermo Fisher Scientific GmbH, Dreieich,
Germany), expelled whey was removed with a Pasteur pipette. The remnant was weighed, and syneresis is expressed as percentage of separated whey related to the initial mass of milk gel.

Microscopy

The microstructure of selected samples was visualised by confocal laser scanning microscopy (CLSM). After rennet addition, samples were transferred into a Nunc Lab-Tek II 4-well chamber (Thermo Fisher Scientific GmbH) at a layer thickness <1 mm, sealed with a cover glass and incubated at 34 °C for 2 h. The gels were cooled at 6 °C for approx. 16 h and examined with a Leica TCS SP5 MP invers (Leica Microsystems GmbH, Wetzlar, Germany) using reflection mode and a 63 x Leica HCX PL APO oil immersion lens. Excitation wavelength was 488 nm (argon laser), and emitted light was detected at 486–490 nm. Pictures were acquired at a resolution of 1024 x 1024 using the Leica LAS AF 2.7.3 software (Leica Microsystems GmbH, Wetzlar, Germany). For image texture analysis, grey level co-occurrence (GLC) matrices (Fagan et al., 2008; Skytte et al., 2015) were generated from grey scale 8-bit images using version 0.4 plugin in Fiji (ImageJ Version 1.49b, www.fiji.sc).

Statistics

Unless stated otherwise, data are expressed as arithmetic mean ± half deviation range (n = 2) or arithmetic mean ± standard deviation (n > 2). All significant statements refer to an error probability level of P < 0.05.

Results and discussion

Gel formation at pH 6.2: influence of dextran and chymosin concentration

Stiffness of rennet-induced milk gels (pH 6.2, 0.04 IMCU mL⁻¹) started to increase after a certain period of time, indicating the onset of the formation of the protein network (Fig. 1). In this study, gelation onset time t_gel is defined as the time where G' exceeds 1 Pa (Jaros et al., 2010), gelation velocity v is the time derivative of the storage modulus dG'/dt and G' was taken 40 min and 2 h after enzyme addition (G'_{40 min} and G'_{2 h}, respectively). In conventional cheese-making, the time span from rennet addition to curd cutting is approx. 30–40 min (Jacob et al., 2011b). t_gel decreased with increasing c_{Dex} from 16.8 ± 0.55 min (reference) to 16.0 ± 1.10 min (c_{Dex} = 7.5 g kg⁻¹) and to 8.9 ± 0.55 min (c_{Dex} = 30 g kg⁻¹) (Fig. 1). Maximum gelation velocity v_{max} increased from 2.46 ± 0.06 Pa min⁻¹ (reference) to 3.42 ± 0.10 Pa min⁻¹ (c_{Dex} = 7.5 g kg⁻¹) to 6.76 ± 0.23 Pa min⁻¹ (c_{Dex} = 30 g kg⁻¹), and the respective time at v_{max} was 30.8 min, 23.9 min and 13.8 min. For the depicted systems, G'_{40 min} was 53.2 ± 2.61 Pa, 70.1 ± 8.1 Pa and 147.6 ± 2.05 Pa, respectively, and G'_{2 h} approached 249.5 ± 4.10 Pa for gels with c_{Dex} = 30 g kg⁻¹.

It is well known that uncharged polysaccharides are able to affect physical properties of acid-induced milk gels (Everett & McLeod, 2005; Mende et al., 2014). We previously described a concentration effect when dextran was added to milk prior to chemical or microbial acidification, and observed an earlier gelation onset and a higher gel stiffness in these systems. This was explained by depletion interactions between casein micelles and dextran (Mende et al., 2013). In rennet-induced milk gels, similar results are reported for guar gum up to a critical concentration (Tan et al., 2007; Galante et al., 2017).

Figure 1 Time-dependent development of storage modulus (G') and gelation velocity (v) (n = 2) in rennet-induced milk gels (0.04 IMCU mL⁻¹, 34 °C) at pH 6.2. Dextran concentration c_{Dex}: Circles, 0 g kg⁻¹; squares, 7.5 g kg⁻¹; triangles, 30 g kg⁻¹.
Increasing dextran concentration of the base milk from 0 to 30 g kg\(^{-1}\) resulted in a continuous decrease of \(t_{gel}\), an increase in \(v_{max}\), and in gel stiffness increase \((G'_{40 \, \text{min}} \, \text{and} \, G'_{2 \, h}; \, \text{Fig.} \, 2)\). This trend is similar for both rennet concentrations, but at 0.04 IMCU ml\(^{-1}\) \(t_{gel}\) was significantly lower and \(v_{max}\) and the stiffness indicators significantly higher because of the more pronounced enzymatic reaction (Zoon et al., 1989; Lucey et al., 2000; Liu et al., 2014; Fox et al., 2017). For instance, \(t_{gel}\) of milk with 0.04 IMCU ml\(^{-1}\) rennet and without dextran was nearly similar to \(t_{gel}\) of milk with only 0.02 IMCU ml\(^{-1}\) but 30 g kg\(^{-1}\) dextran. \(G'_{40 \, \text{min}}\) of gels produced with a higher chymosin level but without dextran was comparable to that of gels made from milk with 15 g kg\(^{-1}\) dextran and 0.02 IMCU ml\(^{-1}\). Stiffness after 2 h showed a more pronounced dependency on \(c_{Dex}\) when 0.02 IMCU ml\(^{-1}\) was used; as a consequence, \(G'_{2 \, h}\) at \(c_{Dex} = 30 \, \text{g kg}^{-1}\) was almost similar.

When milk containing polysaccharides is subjected to rennet-induced gelation, two effects must be considered as follows: (i) depletion interactions between the polysaccharide and casein micelles, and (ii) aggregation of casein micelles caused by enzyme action. During gel formation there is presumably a competition between both effects: At lower polysaccharide concentration enzymatically induced casein coagulation dominates whereas, at higher concentration, polysaccharide – protein interactions become more important. At lower rennet concentration, the rate of \(\kappa\)-casein cleavage is lower, resulting in a reduced gel formation rate, so that the impact of polysaccharides becomes more important. The observations also indicate that advantage can be taken from different structural rearrangements in systems with different amounts of chymosin and/or dextran.

Effect of pH and dextran on gelation behaviour

Gelation profiles of rennet-induced milk gels with pre-adjusted constant pH (6.5, 6.2, 5.9 or 5.7), without or with 30 g kg\(^{-1}\) dextran, are shown in Fig. 3. Without polysaccharide, a lower pH resulted in higher gelation rate and, hence, in a lower \(t_{gel}\) and higher \(G'_{40 \, \text{min}}\). Gel stiffness continued to increase until the end of the measurement, and \(G'_{2 \, h}\) was highest in gels at pH 5.7. Gelation time ranged from 39.8 min (pH 6.5) to 6.9 min (pH 5.7). \(G'_{40 \, \text{min}}\) was ~1 Pa, 50 Pa, 142 Pa and 175 Pa for milk adjusted to pH 6.5, 6.2, 5.9 and 5.7, respectively. During industrial cheese production, the curd is usually cut at a gel stiffness of approx. 20 Pa (Walstra et al., 2006). At pH 6.5 this level of \(G'\) was achieved after ~80 min whereas, at pH 6.2, this time span was reduced to ~30 min. Natural milk pH is approx. 6.7, which decreases during rennet-induced gelation through starter culture activity. The typical pH at cutting is, for e.g. Cheddar, 6.5 but Ong et al. (2012) demonstrated that a lower pH during renneting showed positive effects on cheese texture.

Because of its effect on chymosin activity, pH is one of the most important parameters in rennet coagulation (Fox et al., 2017). Furthermore, milk pH itself affects casein micelle stability. Lowering pH to 5.0 results in micelle destabilization because of (i) dissociation of calcium, (ii) decrease of the charge density of \(\kappa\)-casein, and (iii) reduction of the net negative charge of the caseins and thereby lowering electrostatic repulsion. Consequently, increasing acidity leads to faster gelation and higher gel stiffness (Zoon et al., 1989; Esteves et al., 2003; Ong et al., 2012). Only a few authors reported, however, on rennet-induced gelation at pH < 6 (Zoon et al., 1989; Mellema et al., 2002; Awad, 2007); in their studies, gels at 5.9 or 5.8 were less stiff. In our experiments, gel stiffness increased with pH decreasing to 5.9; a further pH reduction showed only minor effects. Lucey (2002) reported that dissociation of calcium and a decrease in electrostatic repulsion results in a destabilisation of rennet gels. Mellema et al. (2002) ascribed rennet gel weakening at lower pH to a higher rate of rearrangements within the casein network.

The presence of 30 g kg\(^{-1}\) dextran superimposed the impact of milk pH> 5.9 on gelation kinetics, resulting in lower gelation time and higher maximum gelation
rate (Fig. 3). However, at pH 5.7, \( v_{\text{max}} \) was highest (approx. 8.7 Pa min\(^{-1}\)) and independent of whether dextran was present or not. At pH 6.5 and 6.2, gel stiffness was significantly higher for the dextran-containing gels, with \( G'_{40 \text{ min}} \) being 97 Pa and 150 Pa, respectively. At pH 5.9, \( G'_{40 \text{ min}} \) was 142 Pa (without) and 171 Pa (with dextran), but \( G'_{2 \text{ h}} \) was nearly similar (~240 Pa). At pH 5.7 reverse effects were observed: \( G'_{40 \text{ min}} \) was lower for the dextran-containing gels (155 Pa) than for the reference (175 Pa). pH changes do not affect the behaviour of uncharged dextran in solution and its interactions with casein, but they influence casein micelle destabilization because of effects on chymosin activity in the primary coagulation phase (Fox et al., 2017). Consequently, at lower pH, casein aggregation caused by depletion interactions with dextran competes with faster aggregation because of the enzymatic processes. Our results show that, at pH 6.5 and 6.2, dextran-casein interactions are important for gel formation and network stabilization whereas, at pH 5.9, destabilization because of enzymatic casein micelle degradation is more pronounced. At pH 5.7, depletion interactions diminish and dextran additionally imparts casein network formation due to increasing incompatibilities.

The mechanical spectra reflected the typical behaviour of weak gels (data not shown). In the power law relation between \( G' \) and \( \omega \) (\( G' = k \omega^n \)), \( k \) refers to \( G' \) at \( \omega = 1 \text{ rad s}^{-1} \), and the exponent \( n \) reflects time-dependent properties. \( n \) was 0.18–0.19 which is slightly lower than reported in literature (e.g., \( n \sim 0.23–0.25 \); Lucey, 2002), presumably because of differences in milk composition or pre-treatments. For acid-induced gels which usually are more stable than rennet-induced gels, \( n \) is approx. 0.16–0.18 (Lucey, 2002). In this study, \( n \) was higher at lower pH, indicating a more pronounced fraction of viscous contributions to gel strength. This is also evident from tan \( \delta \) at \( \omega = 1 \text{ rad s}^{-1} \), which is lower at higher pH (pH 6.2, tan \( \delta = 0.31 \); pH 5.7, tan \( \delta = 0.34 \)), indicating a more pronounced relaxation of flexible structural bonds (Zoon et al., 1989). However, dextran showed no significant influence on the time-dependent behaviour of the milk gels.

At pH 6.5 and 6.2, CLSM images of rennet-induced gels without or with 30 g kg\(^{-1}\) dextran showed protein networks consisting of thin strands and small pores (Fig. 4). Lower pH during gel formation resulted in more interconnected protein aggregates, thicker strands and larger pores, both for milk gels without dextran (see also Mellema et al., 2002; Esteves et al., 2003; Ong et al., 2012; Liu et al., 2014) but also for the polysaccharide-containing systems. The presence of dextran generally resulted in inhomogeneities and larger pores, but also in denser protein aggregates presumably caused by depletion interactions (Tan et al., 2007). At pH 5.7 we observed the most irregular and least interconnected pores. For quantitative comparison, entropy (ENT) and the inverse difference moment (IDM) were calculated from GLC matrices. ENT is a measure of grey level distribution: high values correspond to images with all shades of grey in a similar amount, which is usually the case for homogeneous structures. ENT ranged between 8.0 and 9.5 for gels without dextran, and was between 6.5 and 7.9 when dextran was present; furthermore, ENT decreased when pH was lower. In both cases, the microstructure changed from being homogeneous with small, evenly distributed pores and thin protein strands to a more heterogeneous one with larger, irregular pores and an increased amount of protein aggregates. IDM is a quantitative measure of the spatial distribution of the grey levels: values are higher in images with few grey level deviations. The higher IDM in milk gels at lower pH and/or the gels containing dextran (Fig. 5) reflects the lower fractions of recurring structural units. The effect of the pH was more pronounced for the dextran-containing gels, where IDM decreased from 0.075 at pH 5.7 (irregular pores and protein aggregates) to 0.052 at pH 6.5 (well distributed pores and protein strands).
Figure 4 Confocal laser scanning microscopy images of rennet-induced milk gels 2 h after chymosin addition (0.04 IMCU mL\(^{-1}\), 34 °C) at different pH without dextran and with 30 g kg\(^{-1}\) dextran.
Forced syneresis $S$ is indicator for hydrogel stability against external forces. $S$ increased with decreasing gel pH, irrespective of whether dextran was present or not (Fig. 5). At a particular pH, however, the presence of dextran is responsible for a significant reduction of syneresis, except for pH 5.7 where $S$ was slightly higher with dextran compared with 24% and 35% without dextran. The most pronounced effects of the polysaccharide are at pH 6.5 and 6.2 where $S$ was $\sim 4\%$ with dextran compared with 24% and 35% without dextran. It is evident from this work that the water binding capacity of polysaccharides such as dextran is a factor significantly contributing to milk gel stability (Tan et al., 2007; Mende et al., 2013).

Influence of continuously dropping pH in presence of dextran

In case of pure milk systems, differences in pH decay rate induced by GDL concentration significantly influenced gelation onset and gel formation rate. The respective measures were $pH_{gel}$ (pH at $t_{gel}$) = 6.19 ± 0.05 ($t_{gel} = 20.35 ± 0.54 \text{ min}$) and $v_{max} = 3.75 ± 0.05 \text{ Pa min}^{-1}$ when pH was continuously reduced from 6.5 to 5.9 within 2 h (Fig. 6), and $pH_{gel} = 6.08 ± 0.01$ ($t_{gel} = 17.35 ± 0.55 \text{ min}$) and $v_{max} = 5.00 ± 0.19 \text{ Pa min}^{-1}$ when pH was reduced from 6.5 to 5.7. Gel stiffness 40 min and 2 h after renneting were higher when acidification rate was higher (final pH 5.7). However, $G'_{40 \text{ min}}$ was significantly lower compared with gels formed at constant pH of 5.9 or 5.7.

Previous literature concluded that combined acid/rennet gelation is a complex process and that various parameters (e.g., temperature, pH, milk pretreatment, casein and enzyme concentration) influence the continuous and synergistic changes of the system (Lucey et al., 2000; Li & Dalgleish, 2006; Le Feunteun & Mariette, 2008; Liu et al., 2014). The continuous reduction of pH during renneting seems to be most important for gel formation processes in combined gels, especially at pH > 5.9 (Le Feunteun & Mariette, 2008; Liu et al., 2014). During acidification, several important physicochemical changes take place before the onset of gelation while, during rennet-induced coagulation alone, there are no major changes up to this point. Le Feunteun & Mariette (2008) explained the higher rate of rearrangements with a reduction in casein particle size and consequently a higher diffusion coefficient. These effects lead to an earlier gelation onset, a faster gelation rate and, consequently, to a higher gel stiffness. Lucey et al. (2000) observed a gelation onset at a relatively high pH between 5.4 and 6.1, depending on GDL and chymosin concentration.
which corresponds to our data. The faster onset of gelation can be also explained with continuing and higher κ-casein proteolysis in combined systems (Li & Dalgleish, 2006).

With dextran present in the gelling system, gelation behaviour significantly changed. As can be seen for milk with 30 g kg$^{-1}$ dextran (Fig. 6), $t_{gel}$ was significantly lower and hence the corresponding pH significantly higher than in systems without dextran. In addition, stiffness of the dextran-containing gels was higher during entire gelation, and $G'_{2\,h}$ was even higher than for gels made at constant pH. Stiffness development was however not affected by pH evolution. The depletion interactions between casein micelles and dextran molecules seem to have the most important impact on the gelation process.

**Concluding remarks**

It is well known that polysaccharides have a certain impact on the formation of milk gels. In the present study, it is demonstrated that dextran, an uncharged homopolysaccharide, affects the gelation process and the properties of rennet-induced milk gels. Adjusting initial milk pH from 6.5 to 5.7 led to a faster enzymatic gelation and higher gel stiffness but also to gels that were more susceptible against external stress.

Information from rheology and CLSM allows a thorough interpretation of the observed effects. In set gels without dextran and at low pH, thicker protein strands and larger aggregates resulted in gels that are stiff but show flexible protein bonds. Nevertheless, gels with higher pH were more prone to structural breakdown by external forces which can be explained by thinner protein strands. Forced syneresis is almost independent of gel stiffness, but presumably affected by pH-dependent microstructural properties and a more open microstructure at low pH.

In the presence of dextran in the base milk and independent of milk pH protein aggregates are larger, and gel stiffness is higher. Syneresis increases when the pH is lower, which results from the larger pores and the disconnected protein structure in the gel. The results demonstrate that dextran is able to stabilise rennet-induced milk gels (pH 6.5, 6.2; higher gel stiffness, more resistant gels) which can be explained by depletion interactions between the polysaccharide and the protein, leading to the formation of larger, more compact casein aggregates. However, the results also show that, at low pH, reverse effects can be triggered: the uncharged dextran is able to destabilise rennet-induced gels at pH 5.7, where the polysaccharide and the aggregated casein micelles seem to exhibit phase separation during gelation, thus resulting in gels with irregular, large pores and very low gel stiffness.

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