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Structure formation in sugar containing pectin gels - Influence of tartaric acid content (pH) and cooling rate on the gelation of high-methoxylated pectin

Abbreviated running title: Structure formation of high-methoxylated pectin in sugar containing gels

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Abstract

The aim of the study was the application of a recently published method, using structuring parameters calculated from dG'/dt, for the characterisation of the pectin sugar acid gelation process. The influence of cooling rate and pH on structure formation of HM pectin gels containing 65 wt% sucrose was investigated. The results show that the structure formation process as well as the properties of the final gels strongly depended on both parameters. With increasing cooling rates from 0.5-1.0 K/min the initial structuring temperature slightly decreased and the maximum structuring velocity increased. The lower the cooling rates, the firmer and more elastic were the final gels. With increasing acid content (decreasing pH from 2.5-2.0) the initial structuring temperatures were nearly constant. The final gel properties varied visibly but not systematically. Gels with the lowest and highest pH were less elastic and weaker compared to those with medium acid concentrations.

1. Introduction

Pectins are branched polysaccharides composed of partially methoxylated polygalacturonic acid. Pectins, isolated from the cell walls of higher plants, are important gelling or thickening agents for the food industry. They are commonly applied in jams and jellies, but they are also used e.g. as stabilisers in acidified milk drinks or as thickeners to improve the viscosity and texture of oil-in-water emulsions. The process of structure formation during gelation is rather complex but its principles are well established and described in the literature (Fraeye, Duvetter, Doungla, van Loey & Hendrickx, 2010). It is generally known that gels are formed and water can be immobilised when junction zones in the smooth regions of pectin molecules form a three-dimensional network via specific intermolecular bonds. Depending on the degree of methoxylation (DM), two main gelation mechanisms are possible. Pectins with a DM above 50% (high-methoxylated, HM) form gels in the presence of saccharides (typically sucrose 55-75 wt%) in acidic environment at pH 2.5-3.5. Their gelling mechanism is a combination of hydrophobic interactions and hydrogen bonds. The low-methoxylated pectin (DM < 50%) network formation is less dependent on pH and soluble solids than the HM pectin gelation. It is promoted by the presence of Ca^{2+}, forming intermolecular ionic junction zones between dissociated carboxyl groups (Fraeye et al., 2010; Thakur, Singh & Handa, 1997; Voragen, Pilnik, Thibault, Axelos & Renard, 1995).

The most common parameters used for investigating the phase transitions in gelling or melting gel systems are the gel point (GP), gel setting time or temperature, melting point, melting time and temperature, respectively. Rheological measurements give the most common and reliable data for the examination of such sol-gel-transitions. The experimental detection of the GP is often described as crossover of storage modulus (G´) and loss modulus (G´´) at a certain frequency (Gigli, Garnier & Piazza, 2009; Stang-Holst, Kjöniksen, Bu, Sande & Nyström, 2006; Iglesias & Lozano, 2004). Lopes da Silva and Rao (2007), however, showed the limitations of this method. The G’-G”-crossover depends on the oscillation frequency as well as on the analytical range of a conventional rheometer, for instance in case of the detection of viscoelastic behaviour in samples with low concentration. Nevertheless, the GP determined this way might be close to the real sol-gel-transition temperature (Lopes da Silva & Rao, 2007). Another method to evaluate pectin systems using oscillation measurements is the structuring development rate (SDR), calculated as dG’/dt (Rao & Cooley, 1993). The research group of the present study suggested additional structuring parameters (Kastner, Einhorn-Stoll & Senge, 2012a, 2012b). The initial structuring temperature (IST) is defined as the temperature at which dG’/dt is different from 0 for the first time, and the critical structuring temperature (CST) is the extrapolated temperature of the first strong increase of dG´/dt.

The structure formation during pectin gelation is an important factor in the production of many food products. In jams with large pieces of fruit, it is important to have rapid setting pectins, to ensure that the structure formation is fast and, thus, to ensure that the fruit pieces are evenly distributed in the product. In the production of clear gels, it is important to have no air bubbles, what can be achieved through the use of slower set pectins as well as through prevention of pre-gelation (May, 2000).

The typical sugar acid gel (SAG) formation of HM pectin is considered to be a two-step process with two types of interactions: The high sugar concentration reduces the pectin-solvent interaction and promotes hydrophobic interactions between the methoxyl groups of the polygalacturonic acid. These interactions dominate at higher temperature. The low pH reduces the dissociation of the carboxyl groups. As a consequence, the electrostatic repulsion between pectin molecules is suppressed and hydrogen bonds can be formed between the non-dissociated carboxyl groups and secondary hydroxyl groups. This mechanism dominates at low temperature (Lopes da Silva & Rao 2007; Thakur et al., 1997; Voragen et al., 1995; Oakenfull & Scott, 1984; Oakenfull & Fenwick, 1977). As a consequence, the most important factors for the HM pectin structuring process and gel properties are intrinsic variables of the pectin molecules (DM, distribution of the ester groups along the pectin backbone, molecular weight, neutral sugar side chains and charge density) as well as extrinsic factors (pH, ionic strength, soluble solids, pectin amount and temperature).

In addition to the pectin type and buffer system, the cooling rate affects the elastic properties of pectin structure formation. Therefore, Rao and Cooley (1993) concluded that the cooling conditions should be controlled to achieve an optimal gel structure. Several rheological studies therefore investigated the viscoelastic properties of pectin systems at different cooling or heating conditions in order to examine the transition from viscous fluids (sol) to elastic solids (gel) or vice versa. Moreover, different pectin, sugar and buffer concentrations of the pectin systems were applied in previous publications. Dahme (1992) investigated the influence of different cooling rates and concluded that the cooling rate of the pectin gel process should be less than 1 K/min to ensure that the structure formation can be detected uniformly without disturbances. Usually, a cooling rate of 1 K/min was used for the investigation of the structuring properties of pectin systems (Almrhag, George, Bannikova, Katopo, Chaudhary & Kasapis, 2012; Agoub, Giannouli & Morris, 2009; Löfgren & Hermansson, 2007; Evageliou, Richardson & Morris, 2000). However, in other studies structure formation of pectin gels was investigated using high cooling rates of 3 K/min (Iglesias & Lozano, 2004; Löfgren, Walkenström & Hermansson, 2002) or low cooling rates of 0.5 K/min or below (Ngouémazong et al., 2012; Fu & Rao, 2001; Rao & Cooley 1993).

Though the influence of the cooling rate on structure formation of pectin sugar gels is generally accepted, the detailed effects were not systematically examined, yet. Furthermore, also the influence of pH on the kinetics of structure formation of HM pectin sugar acid gels has not been investigated by rheological methods in detail, yet. Therefore, the objective of the present study was, to characterise this structure formation of HM pectin sugar acid gels in dependence on (I) different cooling conditions and (II) varying pH by using various tartaric acid concentrations. The structuring process of the gels will be examined by measuring not only the classical gel point but also the recently published structuring parameters initial and critical structuring temperature IST and CST (Kastner et al., 2012a, 2012b) as calculated from rheological measurements.

2. Materials and methods

Two series of experiments were carried out to evaluate the influence of the cooling conditions and of pH on the HM SAG structuring process. In the first series, standard HM SAG formulations were cooled under different cooling rates (0.25, 0.50, 0.75, 1.00, and 2.00 K/min). In the second experimental series, HM SAG compositions of varying tartaric acid concentrations (3 mL: 9.6 mM/kg gel, 5 mL: 15.9 mM/kg gel, 7 mL: 22.3 mM/kg gel, 9 mL: 28.7 mM/kg gel, and 11 mL: 35.1 mM/kg gel) were investigated at a cooling rate of 1.00 K/min.

2.1 Materials

A commercially available high-methoxylated non-standardised citrus pectin with 74.3% galacturonan content, a DM of 69.8% and an intrinsic viscosity of 554 cm^3/g was used for all experiments. The tartaric acid was of analytical grade. Sucrose was food grade and purchased locally.

2.2 Methods

2.2.1 Preparation of the pectin sugar acid gels

The gel composition and preparation based on the empiric method of the IFT Committee (1959). The concentration of tartaric acid was varied from 9.6 to 35.1 mM/kg gel. In all experiments the total concentration of solids was held constant at 65 wt%, including the 0.27 wt% pectin.

The standard procedure for gel preparation was as described below: 2.75 g pectin (0.27 wt%) and 40 g sucrose were dissolved in 430 g demineralised water by stirring. The suspension was heated until boiling, 607.3 g sucrose was added in 3 portions under continuous stirring and the solution was boiled again. While further boiling and stirring, the total mass was reduced to 1020 g. Afterwards, 7 mL (22.3 mM/kg gel) of 48.8 % w/v tartaric acid solution was added in the first experimental series. In the second series the tartaric acid was varied (expected pH range: 2.5 to 2.0): 3 mL (9.6 mM/kg gel), 5 mL (15.9 mM/kg gel), 7 mL (22.3 mM/kg gel), 9 mL (28.7 mM/kg gel), and 11 mL (35.1 mM/kg gel). The whole gel preparation took no longer than 5 min. The respective SAG solutions were poured into jam jars or the preheated rheometer, as described in section 2.2.2 and 2.2.3. The final properties of the SAG solution were within the limits of total solids 64.5 – 65.5 wt%. The total solid was determined by an automatic refractometer (Schmidt and Haensch, Germany).

2.2.2 Determination of a suitable range for the cooling rate

Cooling tests were performed in order to determine cooling rates of SAG with relevance for industrial applications and, thus, to define parameters for the subsequent rheological measurements. The hot SAG solution was filled into jars (200 mL) similar to those used in the food industry for jam production. The lids were closed and the samples cooled down in a bundle at room conditions. The temperatures during cooling and the cooling gradients were recorded in different areas of the bundle and different areas of the individual jam jars. For this, the glasses were arranged in 3 layers, each with nine jars. Moreover, one single jar was investigated in the same way. All measurements in the bundle as well as in the single jar were repeated five times.

2.2.3 Rheological measurements

The viscoelastic behaviour of SAG during cooling was assessed by small deformation oscillation measurements using a rheometer Physica MCR 301 (Anton Paar, Germany) with a profiled rotational cylinder CC27/P1 (diameter 26.66 mm, length 40.01 mm) and Peltier cylinder temperature system TEZ 150P. Hot SAG solutions (section 2.2.1) were transferred onto the pre-heated rheometer (105°C) and the free surface of the samples was coated with silicone oil, the cylinder was closed with a special lid in order to minimise evaporation and cooled to 20°C. The cooling rate was varied in the first experimental series (0.25, 0.50, 0.75, 1.00, 2.00 K/min). In the second experimental series the cooling rate was kept constant at 1 K/min. The dynamic rheological parameters (G' and G'') were recorded during cooling at a frequency of 1 Hz and a strain of 10^{-3}. All SAG were prepared at least three times for each cooling rate as well as for each acid concentration.

From the rheological measurements three temperatures to characterise the pectin structuring process were calculated. The classical GP was defined as cross-over of G' and G'' with $\tan\delta = G'' / G' = 1$. IST and CST were determined as previously described (Kastner et al. 2012a; 2012b) from the structuring velocity, calculated as the first derivation of G' (dG'/dt), using the OriginPro 8.6 software (OriginLab Corporation, USA).

2.2.4 pH measurements

After the rheological measurements the pH was determined in the cooled gel (20 °C) using a Lab850 pH-meter (Schott Instruments) and a special penetration electrode (BlueLine 14pH, Schott Instruments).

2.2.5 Statistical analysis

Analysis of variance was carried out using OriginPro 8.6 software. The Holm-Bonferroni test was used to determine statistically significant differences ($p < 0.05$).

3. Results and discussion

The average cooling rate of the single jar without external cooling was 0.45 K/min, determined in the temperature range of 85-50 °C, in which the phase transition from liquid to solid took place. Cooling rates of the jars in the bundle differed from 0.16 to 0.44 K/min in the same temperature range. In detail, the jars in the middle of the bundle showed an average cooling rate of 0.16 as well as 0.20 K/min and the glasses on the edge of the bundle had a cooling rate of 0.41 to 0.44 K/min. Considering these results, cooling rates for the rheological experiments were selected within this range and above this range in order to determine the effect of cooling rate on structuring process. The structure formation for standard HM SAG in presence of 65 wt% sucrose was investigated at different cooling rates from 0.25 to 2.00 K/min. The structure formation for HM SAG with varying tartaric acid concentration from 9.6 to 35.1 mM/kg gel was investigated at a constant cooling rate of 1 K/min.

3.1 Structuring process as observed for a standard HM SAG

The rheological measurements allowed the characterization of structure formation with respect to the immobilization of water, the determination of the sol-gel transition and the quality of the final gel (Fig. 1a).

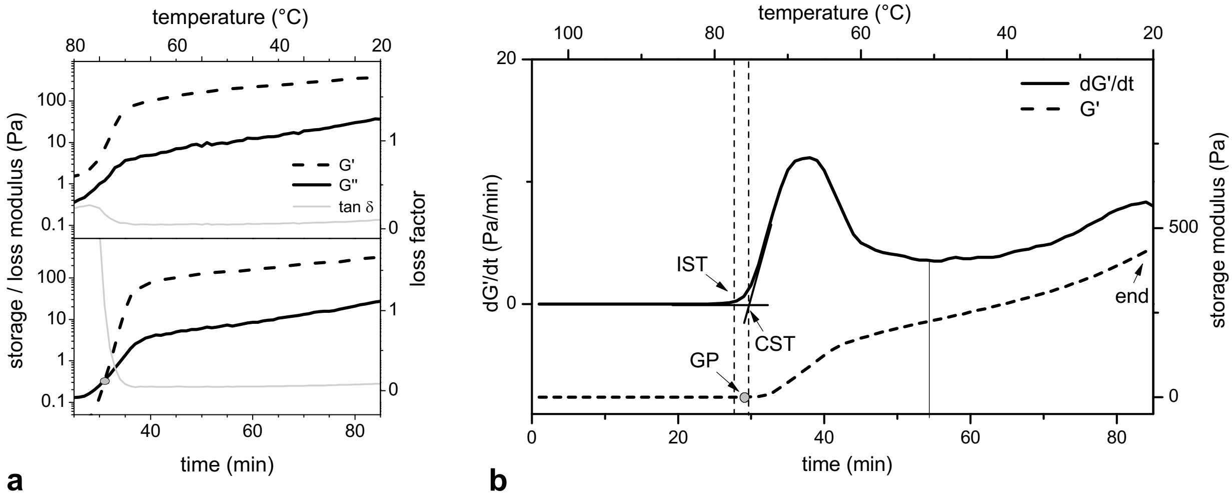

Fig. 1 (a) Oscillation measurements of a HM SAG with acid content of 35.1 mM/kg gel during cooling (1 K/min), G‘ (——, bold line); G“ (- - -), tanδ (—, thin line). Top: without gel point GP, bottom: with GP (●). (b) Typical curve of the structure formation of HM SAG with standard formulation (22.3 mM/kg gel) during cooling (1 K/min); full curve = dG‘/dt, dotted curve = G‘; dotted vertical lines give IST and CST and the start of structuring phases; end: end level at 20°C. The GP is marked as ● on the G‘ curve.

Sometimes in samples cooled at a high cooling rate (2 K/min) or in samples with high tartaric acid content (> 28.7 mM/kg gel), no clear GP could be measured in replicate measurements (Fig. 1a). A similar observation has been described in previous studies, even at low pectin concentration (Gigli et al., 2009; Iglesias & Lozano, 2004; Evageliou et al., 2000; Picout, Richardson & Morris, 2000). In contrast, for all samples it was possible to determine the sol-gel transition range reliably by calculating IST and CST from the first derivation of the storage modulus (Fig. 1b, Table 1, Table 2).

Additionally, often different structuring phases as described by Oakenfull & Fenwick (1977), Oakenfull & Scott (1984), Voragen et al. (1995), Thakur et al. (1997), Fu & Rao (2001), Lopes da Silva & Rao (2007), and Kastner et al. (2012b) could be identified from the structuring velocity curve (Fig. 1b). The phases of structure formation of standard HM SAG, containing 22.3 mM tartaric acid/kg gel and cooled with 1 K/min, are shown in Fig. 1b. The rise in storage modulus as well as structuring velocity was low from 105 to 82°C, nearly no structure formation occurred. With further cooling and after passing the GP (sol-gel transition), the storage modulus increased slowly but continuously and the structuring velocity rose rapidly until 68°C. The initial structure formation was rapid and strong. Below this temperature, a further increase of G‘ but a decrease of the structuring velocity was observed until about 50°C. The gel further solidified at nearly constant structuring velocity. It is known that during the whole gelation process the hydrophobic interactions are weakened (Oakenfull & Fenwick, 1977) and the hydrogen bonds are strengthened (Joesten & Schaad, 1974) with decreasing temperature as described by Alonso-Mougán, Meijide, Jover, Rodríguez-Núñez & Vázquez-Tato (2002), Evageliou et al. (2000), and Oakenfull & Scott (1984). Probably, the phases of dG’/dt indicate these changes of the structuring mechanisms during structure formation from hydrophobic interactions at higher temperatures to hydrogen bonds at lower temperatures. It might be assumed that during gelation the loss of hydrophobic interactions is compensated by an increasing number of hydrogen bonds. Additionally, later on dimer association and inter-dimer aggregation within the pectin gel can take place. In any case, a steady increase of G´ indicates continuous structure formation during the whole cooling process.

3.3 Effect of the cooling rate on structure formation and gel properties

The structuring temperatures GP (77.4 to 71.0 °C), IST (81.6 to 73.0 °C), and CST (75.3 to 71.4 °C) showed a slight but mostly not significant decrease with increasing cooling rates from 0.25 to 2.00 K/min (Table 1). Only the IST of the gels with the highest (2.00 K/min) and the lowest (0.25 and 0.50 K/min) cooling rates significantly differed.

Table 1 Structuring temperatures in dependence on cooling rate (experimental series 1).

cooling rate (K/min)	IST (°C)	CST (°C)	GP (°C)
0.25	81.6 ± 2.6	75.3 ± 2.7	77.4 ± 4.0
0.50	83.6 ± 0.3	74.3 ± 0.5	76.6 ± 0.3
0.75	79.6 ± 0.2	75.1 ± 0.8	74.6 ± 1.3
1.00	78.0 ± 2.4	75.3 ± 0.8	75.3 ± 0.7
2.00	73.0 ± 1.1	71.4 ± 0.9	71.0 ± 1.1

The structuring velocity, however, strongly increased (Fig. 2a). These results can be divided into three different groups:

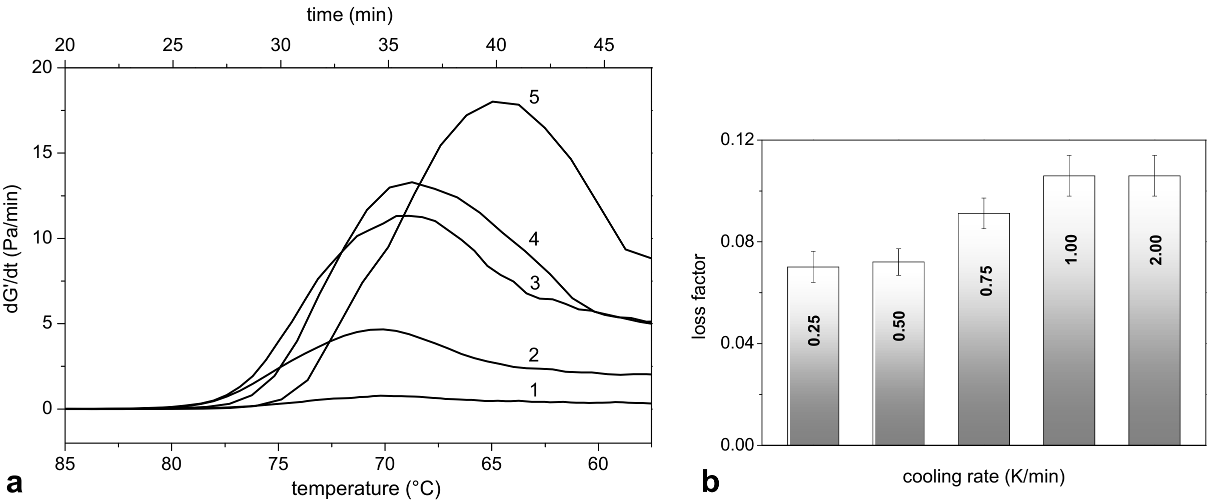

Fig. 2 Influence of cooling rate of standard HM SAG on (a) structuring velocity (dG'/dt): 1: 0.25 K/min, 2: 0.50 K/min, 3: 0.75 K/min, 4: 1.00 K/min, 5: 2.00 K/min; (b) tanδ at the end of the cooling phase at 20°C.

At low cooling rates of 0.25, 0.50 K/min the structure formation of these gels started earlier (higher IST, Table 1) than structure formation in all other gels. It seems that there was sufficient time for an optimum arrangement of pectin molecules to form many strong intermolecular interactions and long junction zones for gelation (Fig. 3). As indicated by low values of the loss factor, after cooling the corresponding gels were more elastic and less viscous than all others (Fig. 2b). At a cooling rate of 0.75 K/min, the maximum structuring velocity of the gels was higher than those of the two lower rates and more similar to those of the third group. The structure formation started, however, earlier and the IST was more comparable to those of lower cooling rates. The final gel was an intermediate one, too. It differs clearly from the slowly cooled gels but only slightly from the rapidly cooled. In gels prepared at high cooling rates of 1.0 K/min or 2.0 K/min the process of structure formation started at lower IST. Probably, independent on temperature a certain time was necessary for the formation of junction zones and for the optimum interaction of pectin chains. The final gels were the least elastic and most viscous and showed a less homogeneous gel structure compared to gels prepared at slow cooling rates. A possible reason is that in the early stage of structure formation less molecular arrangement occurs, and more shorter junction zones or even a certain share of local microgels are formed (Fig. 3), which cause a less homogeneous structure of the SAG three-dimensional network. It is not clear whether the structure formation was already complete after the end of the measurement. Some authors examined pectin gels after the end of the cooling process by continuing rheological measurements and found an aging effect, sometimes referred to annealing (Fu & Rao, 2001; Evageliou et al., 2000; Lopes da Silva & Gonçalves, 1994). This effect may be caused by a transition of shorter to longer junction zones (Fig. 3) as described for gelatin gelation by

Ziegler and Foegeding (1990).

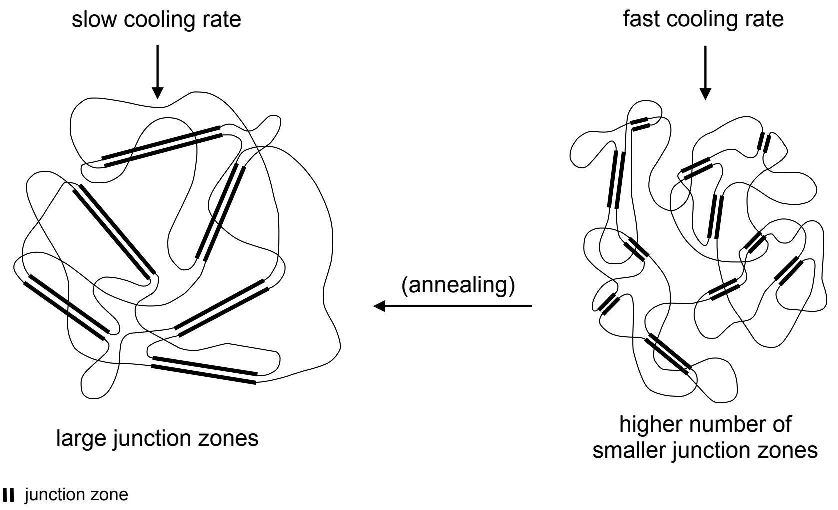

Fig. 3 Formation of pectin junction zones during cooling.

3.4 Effect of tartaric acid content on structure formation and gel properties

Recent atomic force microscopy studies of Fishman and Cooke (2009) have shown that the gel structure was affected by pH and that the distribution of pectin strands and the spaces between strands were relevant factors for evaluation of gel properties like gel strength. For gel formation of HM pectins a low pH is required in order to reduce the dissociation of carboxyl groups and, thus, to reduce electrostatic repulsion. The non-dissociated carboxyl groups can form hydrogen bonds with each other or with hydroxyl groups (Thibault & Ralet, 2003).

Table 2 Structuring temperatures in dependence on tartaric acid concentration (experimental series 2).

tartaric acid (mM/kg gel)	IST (°C)	CST (°C)	GP (°C)
9.6	79.6 ± 2.1	77.2 ± 1.0	76.5 ± 0.5
15.9	79.3 ± 3.1	76.6 ± 0.9	77.0 ± 1.3
22.3	78.9 ± 2.5	75.9 ± 1.2	76.3 ± 1.8
28.7	75.5 ± 2.1	74.9 ± 1.4	74.7 ± 0.6
35.1	75.3 ± 1.7	74.3 ± 1.3	73.0 ± 0.0

In the present study all parameters used for the characterization of structure formation during the gelation process were nearly independent of the acid concentration that was 9.6 mM/kg gel (pH 2.52±0.03), 15.9 mM/kg gel (pH 2.40±0.03), 22.3 mM/kg gel (pH 2.18±0.04), 28.7 mM/kg gel (pH 2.07±0.12), and 35.1 mM/kg gel (pH 2.03±0.08), respectively. All gels showed rather similar structuring temperatures (IST, CST, and GP). The differences from the lowest to the highest concentration of tartaric acid were only about 3 K for each temperature (Table 2). The curves of structuring velocity however, varied to a great extent. At the lowest acid content (9.6 mM/kg gel) structure formation occurred very slowly, probably because at low pH the majority of free carboxylic groups are dissociated and not available for formation of hydrogen bonds (Agoub et al., 2009). Moreover, intermolecular electrostatic repulsion between dissociated carboxyl groups can inhibit structure formation (Evageliou et al., 2000). Gels with moderate addition of tartaric acid (15.9, 22.3, and 28.7 mM/kg gel) showed the highest structuring velocity, their curves were similar (Fig. 4a). These acid contents allowed a rapid undisturbed gel formation. In the gels with the highest acid content (35.1 mM/kg gel) the structure formation was rather slowly, too. In these gels less carboxylic groups were dissociated. The undissociated groups were able to form hydrogen bonds, especially at local “high-acid spots”, and might contribute to pre-gelation and microgel formation (Fig. 4a). Ross-Murphy (1984) described such structures as “incomplete gels”.

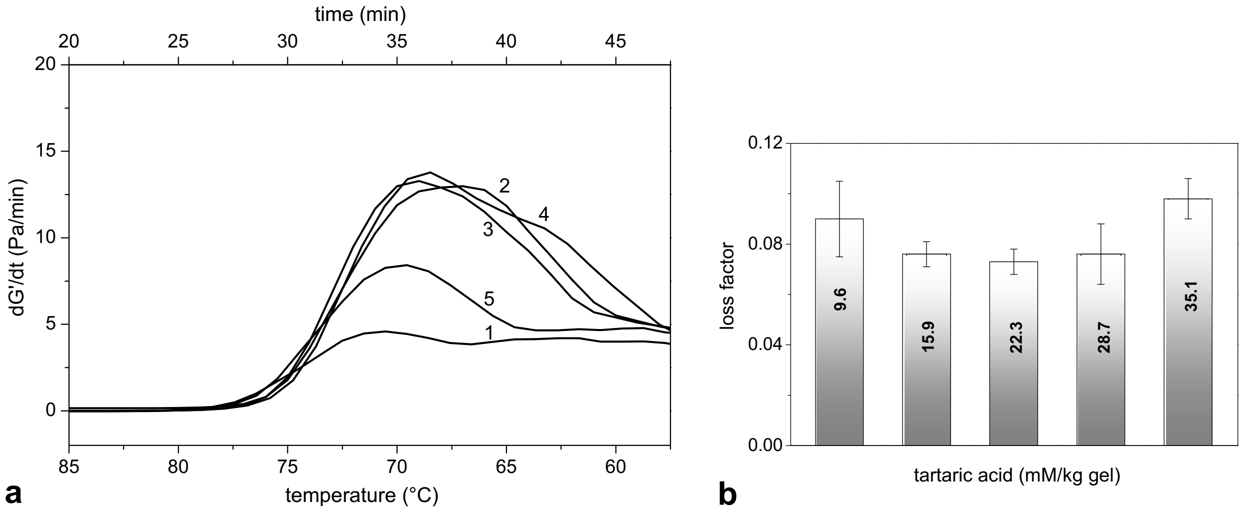

Fig. 4 Influence of acid concentration of HM SAG during cooling (1 K/min) on (a) structuring velocity (dG‘/dt): 1: 9.6 mM/kg gel, 2: 15.9 mM/kg gel, 3: 22.3 mM/kg gel, 4: 28.7 mM/kg gel, 5: 35.1 mM/kg gel; (b) tanδ at the end of the cooling phase at 20°C.

The properties of the final gels with varying acid content differed considerably, too (Fig. 4b). The reasons are similar to those explained above: The gels of intermediate pH were strong, similar to each other and comparable to those of other HM pectins in previous works (Kastner et al., 2012a). At high pH the gels were weaker and more viscous because of the limited number of hydrogen bonds. The “incomplete gels” at the lowest pH were weaker, too because of pre-gelation and microgel formation. A similar effect was discussed for the influence of the amount of calcium ions on the gelation of LM pectins (Kastner et al., 2012b) where a minimum concentration of calcium was required for gelation and a high amount caused microgels, too.

4. Conclusions

The initial structuring temperature IST and critical structuring temperature CST were suitable parameters in order to describe the incipient structure formation for HM sugar acid gels. Even samples with no clear GP, the traditional parameter for characterization of structure formation, could be evaluated this way. Therefore, IST and CST have proved to be valuable additional parameters for the characterization of structure formation and can help to understand the structure formation processes in more detail. Structuring phases could be identified from the shapes of the structuring velocity curves during the gelation process of the HM pectins. It is assumed that they indicate changes of the dominating type of interactions during structure formation.

Structure formation as well as the properties of the final gels strongly depended on the cooling rate. The cooling rate affected both, the structure formation and the SAG structure after cooling. At higher cooling rates, structure formation started earlier and a weaker structure of resulting gels occurred due to a rapid formation of shorter junction zones. In contrast, at low cooling rates a retarded formation of longer junction zones takes place and the final SAG structure is more compact and elastic.

The investigation of the influence of the acid content gave an intermediate optimum range for the structure formation and the final SAG structure at 15.9-28.7 mM/kg gel. Lower as well as higher tartaric acid concentrations were critical and showed a lower structuring velocity and weaker final gels.

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