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Structure formation in sugar containing pectin gels – Influence of Ca2+ on the gelation of low-methoxylated pectin at acidic pH

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Structure formation in sugar containing pectin gels - Influence of Ca^{2+} on the gelation of low-methoxylated pectin at acidic pH

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Abstract

A new method for the examination of the pectin gelation process is presented as a complementation of the most common determination of the gelling point (cross-over of G´ and G´´) from oscillation measurements. It is based on the first derivation dG´/dt from oscillation measurements (named as structuring velocity), and defines an initial as well as a critical structuring temperature. These allow an exact determination of the start of structure formation and description of the structuring process also in gels with pre-gelation that showed no clear GP. Moreover, phases and mechanisms of gelation can be identified and structure developing rates can be calculated.

The application of this method on the gelation of low-methoxylated pectin at pH 3 and 30% saccharose with different contents of Ca^{2+} was tested. The results show differences as well as similarities between the GP and the newly defined structuring parameters that could be partly explained by varying structuring mechanisms at different Ca-content. The initial structuring process started probably with ionic interactions (egg-box junction zones and random crosslinks) via Ca-bridges as well as hydrophobic interactions at temperatures ≥ 60 °C, it was nearly completed around 40 °C. Hydrophilic interactions (below 50 °C) and inter-dimer aggregations (below 25 °C) perhaps dominated the gelation during further cooling. In dependence on the Ca-content, two to three phases could be identified during the structuring process. The properties of the gels after cooling were tested by oscillation measurements as well as the USA-sag method. With increasing calcium content, the elastic behaviour of the gels increased but they became also more and more brittle.

1. Introduction

Pectins are typical gelling agents, traditionally applied in jam and jelly, but used also in other food products such as soft drinks and milk products. The knowledge of the structuring properties and, in particular, the gelling temperature of the pectins is of essential technological importance. The exact determination of this sol – gel transition temperature, i.e. the "gel point" at which the material properties change from more liquid – like to more solid – like, therefore, has been studied for several decades.

Rheological methods, especially oscillation measurements, are often applied and frequently the cross-over of G´ and G´´ with tan δ = G´´/G´= 1 is defined as gel point (Arenaz & Lozano, 1998, Gilsenan, Richardson & Morris, 2000, Löfgren, Walkenström & Hermansson, 2002, Lootens, Capel, Durand, Nicolai, Boulenguer & Langendorff, 2003, Audebrand, Kolb & Axelos, 2006, Stang-Holst, Kjöniksen, Bu, Sande & Nyström, 2006, Gigli, Garnier & Piazza, 2009, Slavov, Garnier, Crepeau, Durand, Thibault & Bonnin, 2009). The method has been developed by Winter & Chambon (1986) initially for chemical gelation. In food products, however, mostly physical gelation via junction zones occurs. Moreover, the point of intersection partly was found to be a function of frequency (Winter & Chambon, 1986, Rao, van Buren & Cooley, 1993, Lopes da Silva, 1994, Lopes da Silva, Goncalves & Rao, 1995, Lopes da Silva & Rao, 2006). Strictly, the cross-over of G´ and G´´ might be defined as gel point only when it is independent on frequency (Stang Holst et al., 2006). Sometimes it might be close to but not identical with the real gel point and therefore is named also "apparent gel point" (Lopes da Silva, 1994, Lopes da Silva, Goncalves & Rao, 1995).

Several attempts have been made to find another method for the gel point definition: researchers from CP Kelco determined the gelling temperature via conductivity (Böttger, Christensen & Stapelfeldt, 2008), and Dobies, Kozak & Jurga (2004) used NMR measurements. Oakenfull & Scott (1984) and O´Brien, Philp & Morris (2009) used relatively simple visual tests. Dahme (1992) and Neidhart, Hannak & Gierschner (1996, 2003) defined a strong decrease of tan δ as an indicator for the gel formation. Grosso & Rao (1998) and Fu & Rao (2001) studied the structuring kinetic of pectin gels and defined the structure development rate SDR = dG´/dt in order to describe precisely the moment, at which the formation of junction zones began. The problem of the gel point determination is, however, not completely solved, yet.

Citrus and apple pectins, isolated from by-products of the fruit juice industry, are the most common pectin types (Rolin, 2002). Their gelling properties vary in dependence on material and environmental factors (Lopes da Silva et al., 2006). Among the material parameters, the number of methoxylated carboxyl groups (degree of methoxylation DM) and their distribution in the polygalacturonic acid backbone (degree of blockiness DB) are very important (Fraeye, Doungla, Duvetter, Moldenaers, van Loey & Hendrickx, 2009, Fraeye, Colle, Vandevenne, Duvetter, van

Buggenhout, Moldenaers, van Loey & Hendrickx, 2010a). Materials with DM > 50% are named as high-methoxylated pectins (HMP) and those with DM < 50% as low-methoxylated (LMP). The typical DM for commercial use is about 60 to 77% for HMP and about 25 to 40% for LMP (Voragen, Pilnik, Thibault, Axelos & Renard, 1995).

The pectin gelation processes are rather complex (Cardoso, Coimbra & Lopes da Silva, 2003). The most important environmental factors are pH, Ca^{2+} and soluble solids (e.g. sugar). They have different and partly opposite effects on the gelling process of different pectin types. High-methoxylated pectins (HMP) form gels in the presence of > 55% soluble solids (mostly about 65% sugar) and at pH < 3.5 (Rolin, 2002, Thakur, Singh & Handa, 1997, Lopes da Silva, 1995). Their gelling mechanism is explained as a combination of hydrophobic interactions, favoured by higher temperature, and hydrogen bonds between undissociated carboxyl groups, dominating at lower temperature (Oakenfull & Fenwick 1977, Oakenfull & Scott 1984). Low-methoxylated pectins (LMP) gel in the presence of Ca^{2+}, forming intermolecular ionic junction zones between smooth regions of neighboured chains (Morris, Powell, Gidley & Rees, 1982, Voragen et. al, 1995, Thakur et al., 1997, Braccini & Perez, 2001). Several studies investigated the special influence of Ca^{2+} and / or pH on the gelling process of LMP in a watery system with no or only small amounts of sugar (Thibault & Rinaudo, 1986, Garnier, Axelos & Thibault, 1993, Gilsenan et al., 2000, Ralet, Dronnet, Buchholt & Thibault, 2001, Braccini & Perez, 2001, Lootens et al., 2003, Cardoso et al., 2003, Dobies et al., 2004, Capel, Nicolai, Durank, Boulenguer & Langendorff, 2005, 2006, Audebrand et al., 2006, Ström, Ribelles, Lundin, Norton, Morris & Williams, 2007, Fang, Al-Assaf, Phillips, Nishinari, Funami & Williams, 2008, Cardenas, Goycoolea & Rinaudo, 2008, Fraeye et al., 2009, Ngouemazong, Tengweh, Fraeye, Duvetter, Cardinaels, van Loey, Moldenaers & Hendrickx, 2011a, Ngouemazong, Kabuye, Fraeye, Cardinaels, van Loey, Moldenaers & Hendrickx, 2011b). Some authors also tested the influence of higher sugar content (Grosso & Rao, 1998, Fu & Rao, 2001, Löfgren et al., 2002, Löfgren, Guillotin & Hermansson, 2006). A current review (Fraeye, Duvetter, Doungla, van Loey & Hendricks, 2010b) gives a good summery of the role of calcium ions in pectins. LMP gelation is favoured by higher pH than that of HMP (above the pectin pK_a 3.5) because the electrostatic interactions via Ca-bridges require a certain number of dissociated carboxyl groups (Thakur et al., 1997, Fraeye et al., 2009, 2010a, b).

In principal, three possible types of junction zones can be formed by LMP: hydrophobic interactions between methoxyl ester groups, hydrophilic interactions between undissociated carboxyl groups and / or hydroxyl groups via hydrogen bonds as well as ionic interactions between dissociated carboxyl groups via Ca-bridges (Figure 1). The latter require a minimum number of 6 to 14 consecutive dissociated carboxyl groups in order to form the typical egg-box structure (Fraye et al., 2010b). In case the total number of such groups is rather low (below the pK_a of pectin), these typical junction zones can be limited. Instead, Ca^{2+} could interact with single dissociated carboxyl groups in

an undissociated neighbourhood, forming monocomplexes by charge reversal on a single chain or random (unspecific) crosslinking between separate chains (Siew & Willams, 2005, Fang et al., 2008). Though these complexes are no typical Ca^{2+} -junction zones, they additionally reduce the electrostatic repulsion, possibly create even electrostatic attraction and, in any case, promote a closer contact of the pectin molecules and support gel structure formation. Similar effects are described also by Cardoso et al. (2003), Fraeye et al. (2009) and Ngouemazong et al. (2011a).
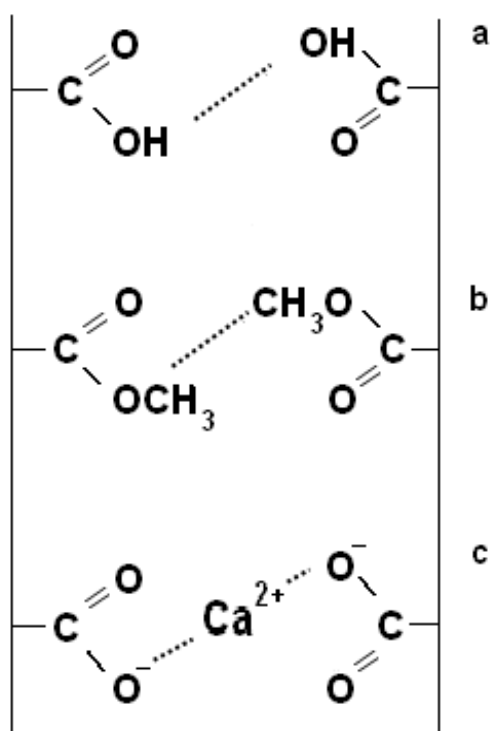

Figure 1   Structure formation mechanisms in pectin gelation. A hydrogen bonds between undissociated carboxyl groups, b hydrophobic interactions, c random ionic interactions (crosslinks) between dissociated carboxyl groups. Ca-bridges at subsequent free dissociated carboxyl groups can form egg-box junction zones as known from many references (e.g. Braccini et al., 2001).

Electrostatic repulsion between pectin molecules is low at pH < pK_a because of a high share of undissociated carboxyl groups. This favours association and aggregation of pectin chains by intermolecular hydrogen bonds and additionally stabilises these systems in the absence of calcium (Gilsenan et al., 2000, Cardoso et al., 2003). Moreover, Cardoso et al. (2001) and Cardenas et al. (2008) assume that, after initial dimer formation, also inter-dimer interactions and cross-linking of pectin molecules occur by associations of threefold helices with contributions of hydrophobic interactions and hydrogen bonds.

In contrast to HMP, high sugar content is not essential for LMP gelation but could support it by binding water and promoting close contact of neighboured molecules.

Altogether, the typical calcium-mediated LMP gelation can be seen as a two-step process: After initial dimerisation by strong electrostatic interchain associations via calcium ions with contributions of hydrophobic interactions at high temperature and hydrogen bonds at lower temperature, follows a subsequent aggregation of dimers that additionally increases gel strength (Lopes da Silva & Rao, 2006, Cardenas et al., 2008).

The aim of the presented paper is (I) to present a new method for the characterisation of the pectin gel structuring process by using the first derivation of the storage modulus dG´/dt from oscillation measurements and (II) to apply this method for the investigation of calcium influence on gelation of LMP at pH 3.0 and in the presence of 30% saccharose.

2. Materials and methods

The gel composition and preparation is based on a method that is applied in the pectin industry for testing the gelling properties by the Ridgelimeter (USA-sag method, IFT Committee, 1959) according to Cox & Higby (1944). The quantities were slightly modified in order to get the necessary amount of pectin solution. All experiments were made four times, the control tests without calcium in duplicate.

2.1. Materials

The pectin was a commercial low-methoxylated non-standardized citrus pectin with 81.5% galacturonic acid content, DM 30.2% and intrinsic viscosity = 336 cm^3/g. Citric acid, tri-sodium citrate dehydrate and calcium chloride dehydrate were of analytical grade (Sigma-Aldrich), saccharose was of food quality from a local supermarket.

2.2. Methods

Gel preparation

637.5 g demineralised water, 7.5 ml 54.3% w/v citric acid solution and 15 ml 6% w/v sodium citrate solution were mixed in a steel pot and 6 g dry pectin powder, mixed with about 40 g saccharose, was added while stirring. The suspension was heated quickly until boiling, 224 g saccharose was added in 3 portions and the solution was boiled again. Afterwards, the required amount of 2.205% w/v calcium chloride dehydrate solution (25 / 31 / 37.5 / 44 / 50 ml, respectively) was added and while further boiling and stirring the total mass was reduced to 900 g. The whole process should take no more than about 5 min.

The stochiometric ratio between calcium and carboxyl content R = 2Ca^{2+}/COOH was 0.46 / 0.58 / 0.70 / 0.82 and 0.94, respectively. This means 0.42 / 0.52 / 0.62 / 0.73 and 0.83 mM CaCl$_2$ per 100 g gel. The regular amount of CaCl$_2$ in the standard procedure is 37.5 ml (0.62 mM/100 g gel, R 0.70); variations were made in both directions.

Rheological measurements

The applied rheometer was a Physica MCR 301 (Anton Paar, Germany). Oscillation measurements (temperature sweep) of storage modulus G´ and loss modulus G´´ were made using a double gap rotational cylinder CC27/P1 with Peltier cylinder temperature system TEZ 150P. Samples were transferred onto the pre-heated rheometer (100 °C) and cooled to 10 °C with a cooling rate of 1 K/min. The sample was coated with silicone oil and the cylinder was closed with a special lid in order to avoid evaporation. Dynamic rheological parameter (G´ and G´´) were recorded during cooling at a frequency of 1 Hz and a deformation amplitude of γ 0.001.

Ridgelimeter (USA-sag method)

This method is rather empirical but frequently used for routine tests in the pectin industry, yet. The hot pectin solution was filled into three special glasses which were stored at 25 °C for 24 h before measurement. The single gels were removed carefully from the glasses and transferred on a plate. The percentage of sagging of the gel cone under its own weight within 2 min is measured. From this value the °SAG can be calculated.

pH

The pH was determined in the gel after Ridgelimeter measurement using a Lab850 pH-meter (Schott Instruments) and a special penetration electrode.

Examination of structure formation

From the G´ data, the first derivation was calculated and smoothed using Origin 8.1 software. Two characteristic temperatures were determined from this first derivation as shown in Figure 2. The *initial structuring temperature IST* is the temperature at which the value dG´/dt was 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 *average structure developing rates SDR$_a$* was calculated from differences of storage moduli during cooling time for the total gelling process:

$$SDR_a = \frac{G'_{end} - G'_{IST}}{t_{end} - t_{IST}}$$

G´$_{IST}$ and t$_{IST}$ are parameters at the initial structuring temperature IST and G´$_{end}$ and t$_{end}$ are the final values at 10 °C.

3. Results and discussion

3.1. Structure formation parameters

Rheological tests of the gelling behaviour of pectins sometimes give no clear (apparent) "gel point" (GP) as a cross-over of G´ and G´´. Either G´ may be higher than G´´ already from the start of the rheological measurements, or the curves are more or less parallel during a longer cooling period without a clear intercept. The difficulties of studying pectin gelation in general are discussed by Lopes da Silva & Rao (2006) and those of LMP by Fraeye et al. (2010a, b). Therefore, an additional method might be helpful in order to describe the structure formation process.
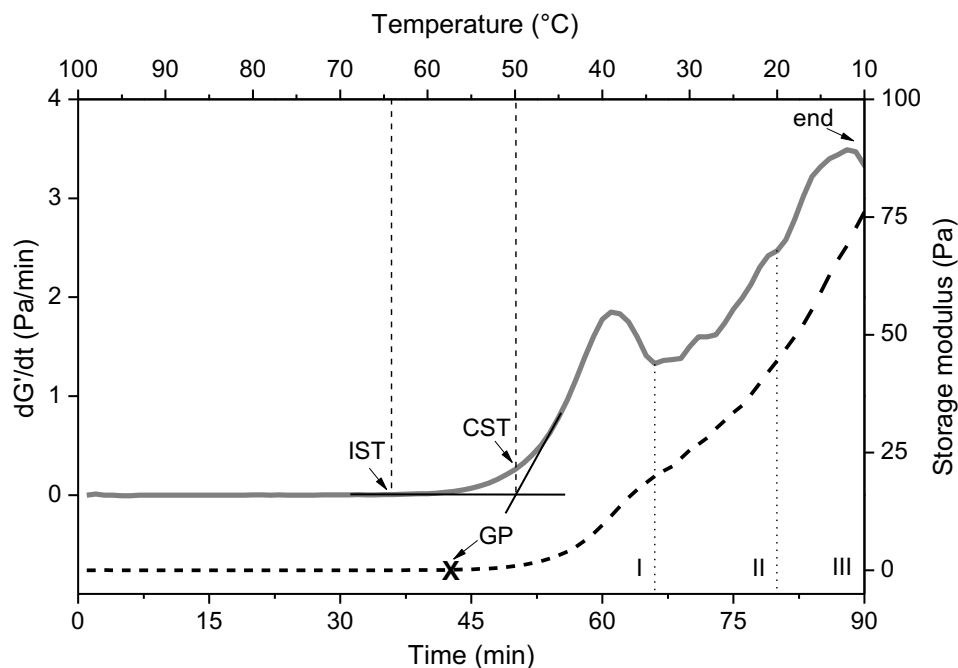

Figure 2 Evaluation of the first derivation dG´/dt in a gel with 0.62 mM CaCl₂. Full curve = dG´/dt, dotted curve = G´; vertical lines give IST and CST (---) and the start of structuring phases (····); IST = initial structuring temperature and CST = critical structuring temperature; end = end level at 10 °C. The GP is marked as X on the G´ curve.

The first derivation dG´/dt was used already for the description of the gelling kinetic of pectins and calculation of the structure developing rate SDR (Grosso & Rao, 1998, Fu & Rao, 2001, Cardoso et al., 2003). dG´/dt can be seen as *structuring velocity* and changes of this velocity are indicators for the start of structuring process as well as for further alterations (phases) during cooling. After smoothing the first derivation of the G´ curve the new parameters *initial structuring temperature IST* and the *critical structuring temperature CRT* (Figure 2) were determined. The IST is an indicator for the start of structure formation and the CRT for a first acceleration in structure formation. These two temperatures could be found for any pectin we have ever studied, not only in the experiments described in this paper. Therefore, this method seems to be a good alternative or complement for the classical "gel point", defined as cross-over of G´ and G´´.

3.2. Application of the structure formation parameters temperature on the gelation of LMP

The characteristic temperatures defined above shall be applied for the discussion of the gelation of LMP at pH about 3 and with 30% saccharose in the presence of different amounts of Ca^{2+}.

Typically, gelation of LMP is dominated initially at high temperature by formation of egg-box junction zones via calcium-bridges and hydrophobic interactions. During further cooling, the influence of hydrogen bonds should increase, supported by interchain inter-dimer associations. Random electrostatic interactions of Ca^{2+} with single dissociated carboxyl groups of pectin chains (calcium crosslinking) could promote the structuring process.

In case of the applied pectin system, some divergences from this typical behaviour were expected: the number of methoxyl groups was rather low at 30% DM but should not be ignored; the high starting temperature of the measurements (100 °C) is favourable for hydrophobic interactions. The free carboxyl groups were assumed to be randomly distributed as it is typical for most commercial pectins after chemical demethoxylation (Fraeye et al., 2010b, Ngouemazong et al., 2011a). The number of dissociated free carboxyl groups should be relatively low at pH about 3 (below the pK_a 3.5). Therefore, it was expected that the formation of typical egg-box junction zones would be limited and more interactions between undissociated carboxyl groups via hydrogen bonds would be formed instead. The number of random (unspecific) calcium crosslinking via single dissociated carboxyl groups should increase with rising Ca^{2+}. The high sugar content in the gels could additionally promote the interchain interactions as it is known from HMP gelation.
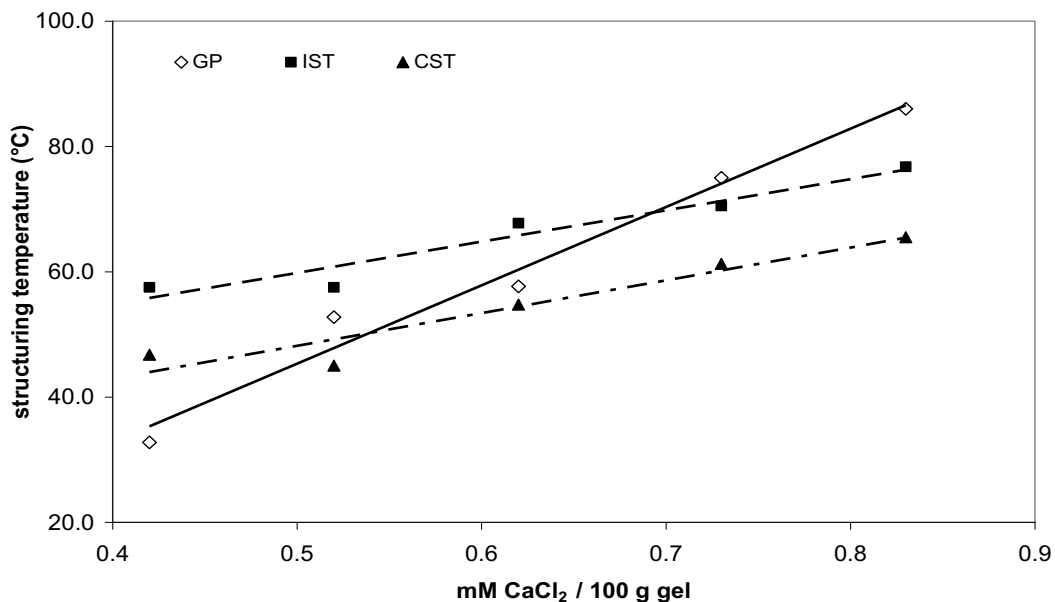

Figure 3 Comparison of the structuring temperatures in dependence on calcium content. GP = gel point = intersection of
G′ and G′′ (—); IST = initial structuring temperature (---); CST = critical structuring temperature (- • -).

As can be seen from Figure 3, the gel point temperature as well as the initial and the critical structuring temperatures increased at higher calcium content. This confirmed the crucial role of Ca^{2+} for the gelling of LMP (e.g. Grosso & Rao, 1998, Lootens et al., 2003, Cardoso et al., 2003, Cardenas et al. 2008, Fraeye et al., 2009, 2010a, b). IST and CST developed in a nearly parallel way; IST was always about 10 K higher than CST. The gel point temperature GP behaved different: at low calcium concentration (0.42 mM, R=0.46) it was found about 25 K below the IST and also CST, and at high calcium content (>0.73 mM, R>0.82) GP was above IST. It should be considered, however, that at high calcium content the GP could not be determined for all samples (Table 1) and the values were therefore rather vague. This will be discussed in detail below.

Table 1   Data of structure formation. $CaCl_2$ is given as mM content in the final gel mass of 900 g as well as R = stoichiometric ratio Ca^{2+} / COO^-; GP = gel point = intersection of G´ and G´´; tan δ_{end} = loss factor at 10 °C; IST = initial structuring temperature; CST = critical structuring temperature; M = mean value

	CaCl₂		gel properties		G´ and G´´				dG´/dt	
	mM /	R	pH	° SAG	G´ 10 °C	G´´ 10 °C	tan δ_{end}	GP	IST	CST
	100 g gel				Pa	Pa		°C	°C	°C
1	0	0	3,26		0,05	0,5	10,0		19	18
2	0	0	3,34		0,05	0,51	10,2		21	21
M	**0**	**0**	**3,3**		**0,05**	**0,51**	**10,1**		**20**	**19,5**
1	0,42	0,46	3,13		6	2	0,415	30	57	46
2	0,42	0,46	3,17		8	3	0,333	34	56	47
3	0,42	0,46	3,18		7	3	0,374	33	59	47
4	0,42	0,46	3,18		7	3	0,369	34	58	47
M	**0,42**	**0,46**	**3,17**		**7**	**2,75**	**0,373**	**32,8**	**57,5**	**46,8**
1	0,52	0,58	3,12	13,07	28	5	0,172	55	56	50
2	0,52	0,58	3,12	30,11	35	5	0,159	50	60	42
3	0,52	0,58	3,21	24,50	33	5	0,162	51	58	40
4	0,52	0,58	3,15	42,26	25	4	0,177	55	56	38
M	**0,52**	**0,58**	**3,15**	**27,49**	**30,25**	**4,75**	**0,168**	**52,8**	**57,5**	**42,5**
1	0,62	0,70	3,13	100,68	73	11	0,138	58	71	50
2	0,62	0,70	3,03	101,34	81	12	0,133	57	64	54
3	0,62	0,70	3,10	100,84	97	13	0,128	58	67	57
4	0,62	0,70	3,11	95,50	105	12	0,127	58	69	58
M	**0,62**	**0,7**	**3,09**	**99,59**	**89**	**12**	**0,132**	**57,8**	**67,8**	**54,8**
1	0,73	0,82	3,07	127,38	219	26	0,121	75	70	61
2	0,73	0,82	3,06	127,79	216	29	0,134		74	63
3	0,73	0,82	3,07	142,24	213	26	0,118		70	61
4	0,73	0,82	3,08	138,96	206	25	0,127		68	60
M	**0,73**	**0,82**	**3,07**	**134,09**	**213,5**	**26,5**	**0,125**	**75**	**70,5**	**61,25**
1	0,83	0,94	3,07	146,82	348	40	0,121	80	76	65
2	0,83	0,94	3,09	150,96	397	48	0,121		78	66
3	0,83	0,94	3,06	156,37	395	47	0,119	92	80	65
4	0,83	0,94	3,06	150,50	345	42	0,120		73	66
M	**0,83**	**0,94**	**3,07**	**151,16**	**371,25**	**44,3**	**0,120**	**86**	**76,8**	**65,5**

The detailed discussion of the gelling process with respect to possible structuring mechanisms at different Ca-contents is illustrated by Figure 2, 4 and 5 a-d. The Figures 2 and 5 are single measurement curves, the data of IST and CST in the text are medium values of four repeated measurements. For reproducibility see Figure 4 and Table 1. Some of the structuring velocity curves allowed the hypothesis of a two- or three-phase gelling process (Figure 4). Two phases of gelation are also described by Fu & Rao (2001), the according temperature ranges and activation energies for the first (70 to 50 °C) and second phase (50 and 20 °C) varied in different pectins.
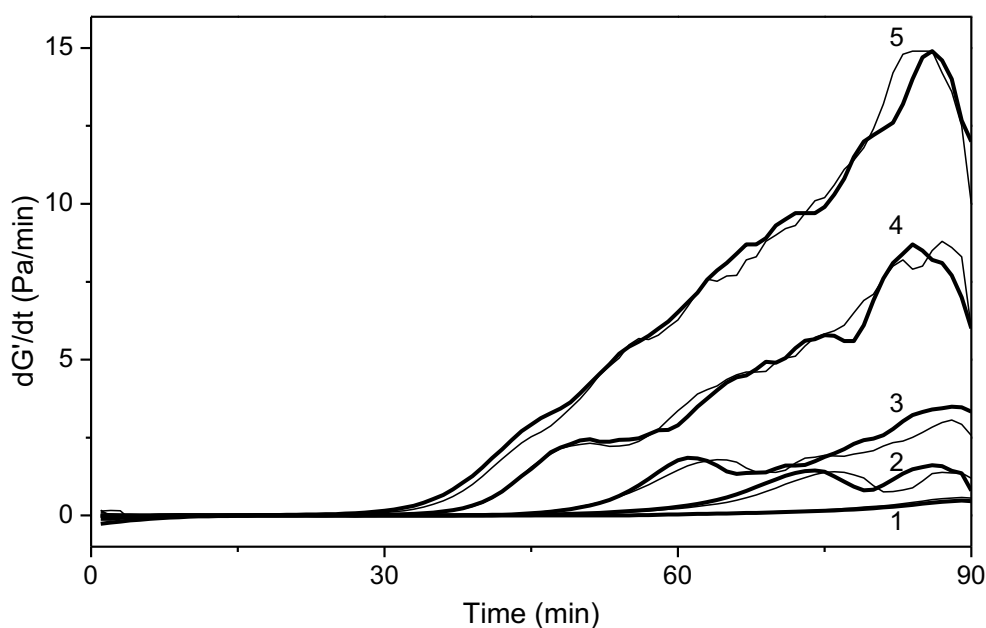

Figure 4   Structure velocity dG´/ dt in dependence on calcium content in 100 g gel: 1 0.42 mM, 2 0.52 mM, 3 0.62 mM, 4 0.73 mM, 5 0.83 mM.

i. Mixtures without any calcium did not gel at all but started a kind of "structure formation" below 20 °C (Table 1). A possible explanation gives Ngouemazong et al. (2011a) who suggest a gel-like characteristic in concentrated LMP systems in the absence of calcium and without junction zone formation. Even a high share of sugar, that should allow a gelation of LMP also under these conditions as described by Gilsenan et al. (2000) and Cardoso (2003), had no real gel-promoting effect.

ii. A low number of Ca^{2+} (0.42 mM / 100 g, R 0.46) were already sufficient to initiate a certain gel formation as can be seen in comparison to (i). A continuous structuring process started at IST 57 °C (Figure 5a) probably with a small number of ionic egg-box and / or crosslinking via Ca^{2+} as well as by hydrophobic interactions. They were, however, not strong enough for a real gelation. With further cooling increasing formation of hydrogen bonds started (Gilsenan et al., 2000, Cardoso et al., 2003) and the structuring process was accelerated despite of the decreasing influence of hydrophobic interactions. This is indicated by the CST 47 °C.

Additionally, dimer associations could become more important with decreasing temperature. A low GP of 32 °C below CST confirmed the transition from the liquid-like to a dominating solid-like system.
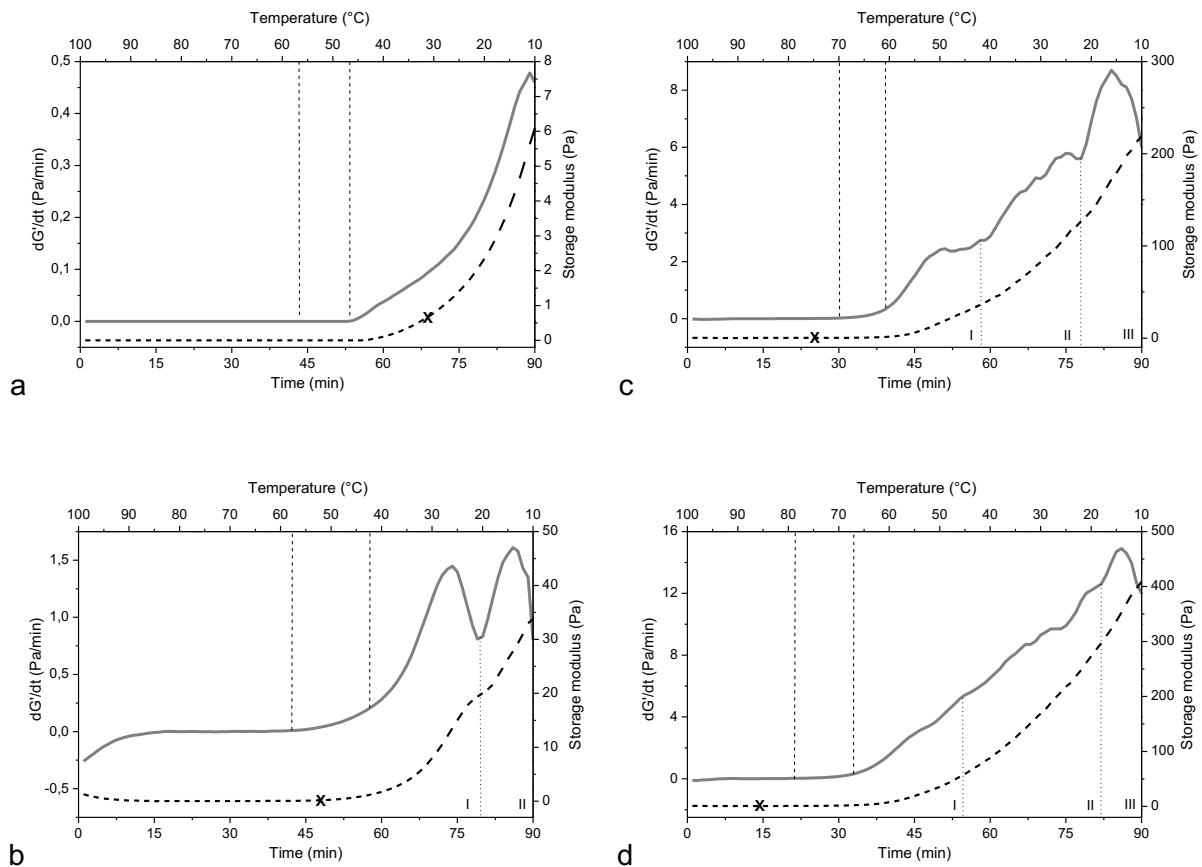

Figure 5   Diagrams of gels with different calcium content in 100 g gel: a 0.42 mM, b 0.52 mM, c 0.73 mM, d 0.83 mM; full curve = dG´/dt, dotted curve = G´; vertical lines give IST and CST (---) and the start of structuring phases (·····); X on the G´ curves marks the GP. For 0.62 mM/100 g gel see Figure 2.

iii.   At higher Ca^{2+} concentration (0.52 mM / 100g, R 0.58) IST and CST were comparable to (ii) but this time the GP at 53 °C was found already shortly after IST 57 °C (Figure 5b). Two clear phases could be defined: phase 1 started from IST, probably with formation of egg-box and other ionic interactions as explained by Cardoso et al. (2003), Siew & Williams (2005) and Fraye et al. (2009) as well as hydrophobic junction zone. It was strongly supported by hydrogen bonds (especially below CST 42 °C) as well as by high sugar content and accelerated with increasing contact between pectin chains during cooling. The second phase started rather late at about 20 °C and could be possibly ascribed mainly to increasing dimer associations and inter-dimer aggregations.

iv.   The structuring process of gels containing 0.62 mM $CaCl_2$ / 100 g (R 0.7) began earlier than in (iii) at IST 67 °C (Figure 3), the higher calcium content obviously accelerated the structure formation considerably by formation of more ionic interactions. The GP 58 °C was found again between IST and CST (55 °C), but on a higher level than in (iii). This time even three phases

could be identified in the structuring process. It was assumed that ionic junction zones via Ca-bridges together with hydrophobic interactions dominated the first phase, and that hydrogen bonds became a supporting force in the second. This second phase seemed to be partly comparable to the first one of (iii). The transition from phase 1 to 2 was near 40 °C. Dimer interactions and inter-dimer aggregations could be ascribed to a third phase below 20 °C, comparable to phase 2 of the gels in (iii).

v. With further increasing calcium content of 0.73 mM / 100g (R 0.82), the determination of the gel point was possible only for one of four samples and the resulting value was, therefore, rather vague. In contrast, IST and CST could be determined without any problems but this time below GP (Figure 5c). It seems that partly pre-gelation (formation of micro-gel particles) happened already during the preparation process (no GP found by temperature sweep tests) or immediately after preparation in the starting phase of the measurements (GP > IST), though the pectin mixtures seemed to be homogenous, yet. A similar effect was described by Morris (2009). He defined a "weak gel structure", formed by some egg-box junction zones rapidly after gel preparation and a "true gel structure", developing on cooling by other mechanisms. The micro-gels of the "weak gel structures" seemed to be irregular solid particles that caused an "apparent gel point" but a real network was formed later during further cooling. The structuring process of the tested pectin system could be divided into three phases again, the first below IST 70 °C with acceleration at CST 61 °C, the second beginning at around 40 °C and the third below 22 °C. The structuring processes in these phases were assumed to be comparable to those explained in (iv).

vi. At the highest calcium content (0.83 mM / 100 g gel, R 0.94) less clear structuring phases could be defined and the GP, found already at 86 °C, was as vague as in (v). The "true" gelling process (phase 1) started at IST 77 °C and was accelerated at about 65 °C (CST). The high number of Ca^{2+} probably was able to increase the structuring velocity by forming more ionic interactions that were dominating the whole gelling process. Some small peaks or shoulders below 45 °C could be perhaps ascribed to the supporting effect of hydrogen bonds but formed no single second phase. The clear peak below 20 °C, resulting from increasing dimer interactions, corresponds again to phase 3.

Altogether, during gelation of LMP with different Ca-content two to three phases were found with differing starting and final temperatures. The first phase could be ascribed to ionic egg-box and random crosslinks together with hydrophobic interactions; it was clearly detected in gels with more than 0.52 mM $CaCl_2$ / 100 g and shifted to higher temperature with increasing Ca^{2+}. The second phase (in gels with less Ca^{2+} also found as first one) perhaps indicated the contribution of hydrogen bonds and the third phase (in 2-phase processes the second one) might be dominated by inter-dimer interactions. At the highest tested Ca-content, the ionic interactions seem to dominate the

whole gelling process with no clear difference between phase 1 and 2. Figure 6 shows a possible model of the structuring process.
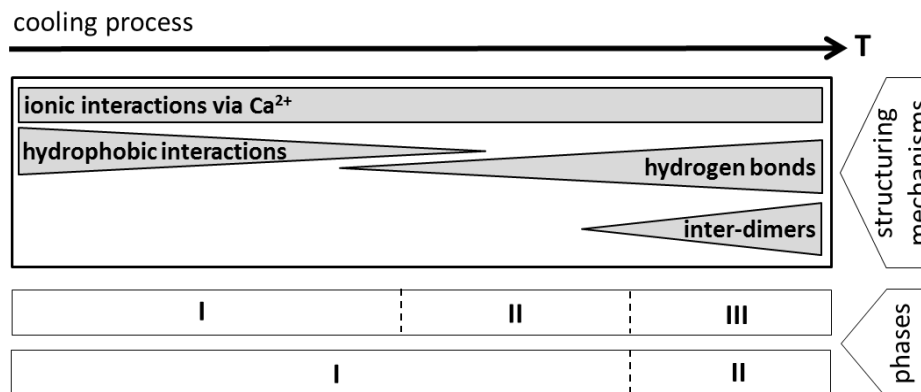

Figure 6   Model scheme of the structuring process of the tested low-methoxylated pectin system during cooling for 3 phases or 2 phases process.

3.3. Gel structure after cooling

The question is, whether the calcium content influenced not only the gelation process but also the final gel structure. This will be discussed considering different gel parameters.

The final values of G´ and G´´ after cooling at 10 °C as well as the Ridgelimeter tests gave information about different gel properties. All three parameters strongly correlated with the increasing Ca-content (Table 1 and Figures 7, 8).

The °SAG value is characteristic for the ability of a gel to keep its shape under its own weight (gel form stability). The samples with no Ca^{2+} did not really gel and could not be measured. Those with low content (0.42 mM) were rather weak and deformed quickly within the 2 min measuring time, it was impossible to get results by this method. The gels became stiffer and less sagging at higher Ca-content and, moreover, more and more brittle. The increase of °SAG was not linear and, altogether, moderate (the highest value was about six times higher than the smallest (Figure 7).
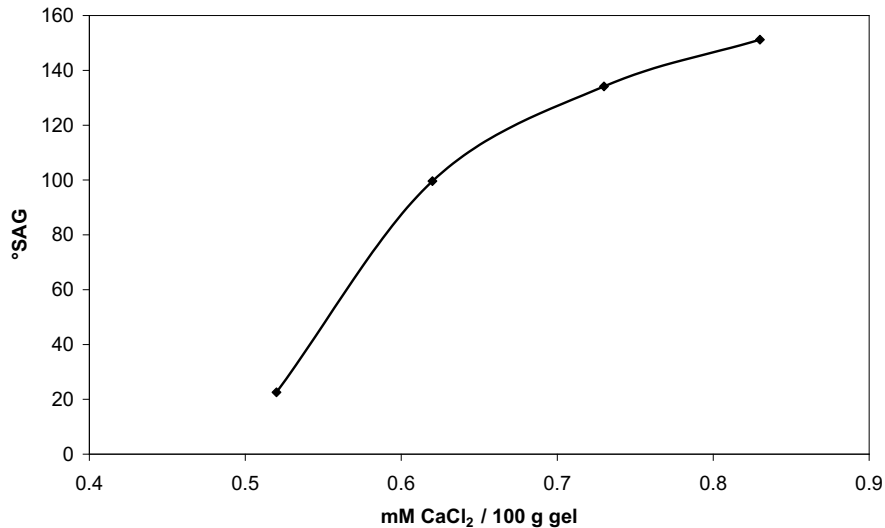
Figure 7   Influence of the calcium content on the gel form stability (°SAG) in the Ridgelimeter method.

The storage modulus at the end of cooling (G´$_{end}$) characterised the elastic material properties of the samples. These increased with higher content of Ca^{2+} (maximum increase factor > 50, Figure 8), which made the gels more stable and elastic. That was found also by Ngouemazong et al. (2011a) for gels without sugar.
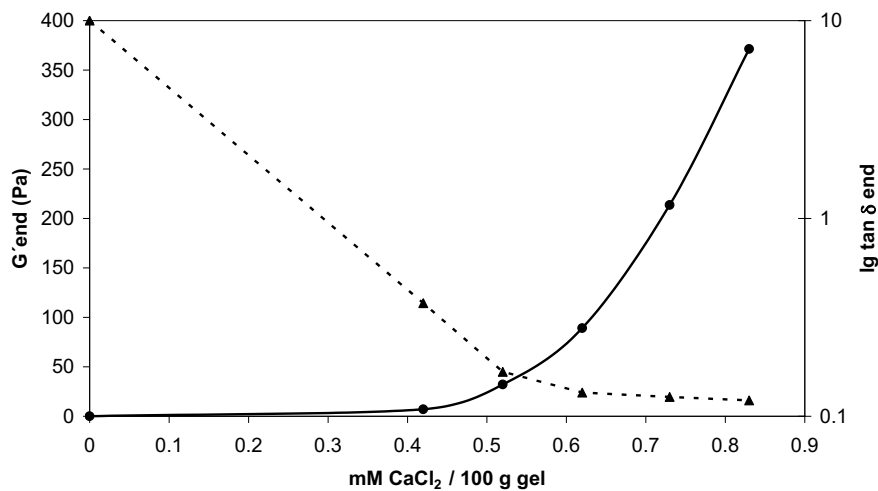

Figure 8   Influence of the calcium content on the storage modulus G´ (full line) and the loss factor tanδ$_{end}$ (dotted line) at the end of the cooling phase at 10 °C.

The influence of the loss modulus G´´ can be found in the loss factor tan δ = G´´ / G´ (Figure 8). Though G´´ also increased with higher Ca^{2+} (factor about 15), the differences were not as high as in case of G´ and thus G´ dominated. After complete cooling, tan δ_{end} was the highest in mixtures without any calcium (about 10, Table 1), confirming their dominating liquid-like viscous material properties. With increasing Ca^{2+}, tan δ_{end} decreased and above 0.62 mM it was nearly constant.

These gels were more solid-like and elastic but also increasingly brittle as found already during the Ridgelimeter tests. A comparable effect of the calcium concentration on gel properties was found also by Cardenas et al. (2008), Fraye et al. (2010a) and Ngouemazong et al. (2011a).

The varying properties of the final gels confirmed the assumption of varying gel structures, resulting from different structuring mechanisms in dependence on the calcium content. Systems with low Ca-content, gelled mainly by non-ionic interactions, were very weak and deformable. Gels with a higher (optimum) amount of Ca^2, formed by a combination of ionic and other interactions were form-stable and rather elastic. Gels with the highest concentration of Ca^{2+}, dominated by ionic structuring mechanisms and showing pre-gelation, were brittle and susceptible to mechanical destruction.

3.4. Structure developing rate

The average structure developing rates SDR_a was calculated for the total gelation process (Figure 9). It increased strongly and non-linear with the calcium contents of the gels and confirmed the well-known role of Ca^{2+} for the gelation of low methoxylated pectins.
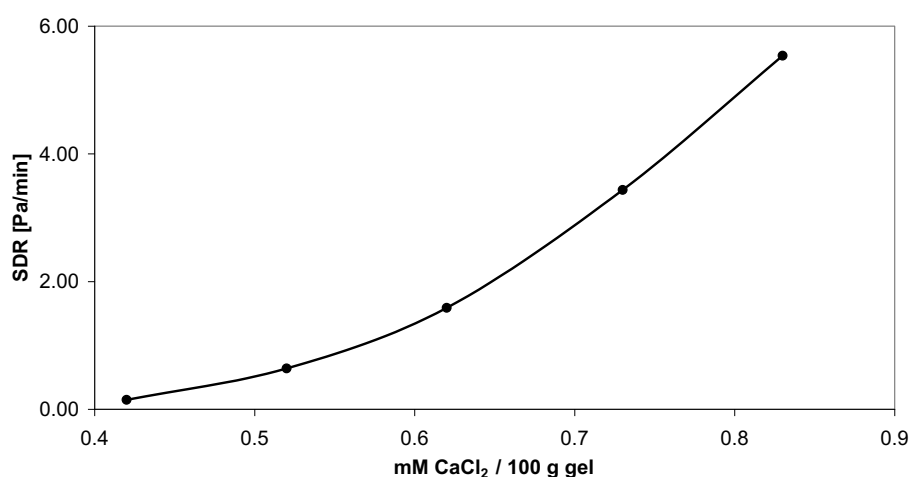

Figure 9   Influence of the calcium content on the structure developing rate during the whole structure formation (SDR_a).

4. Conclusions

i.   The application of the first derivation dG′/dt, the structuring velocity curve, supports the understanding of the gelation process. The suggested new parameters initial structuring temperature IST and critical structuring temperature CST do not describe the same event in pectin gelation like the widely used cross-over of G′ and G′′ (gel point GP). The IST was higher than the GP as long as the latter could be determined regularly. In these cases, IST can be seen as the beginning of the structuring process in a system with dominating liquid-like character. The CST of the tested gels was found mainly below the GP. It seems that the structuring process was strongly accelerated after a certain critical number of junction zones had been formed in an increasingly solid-like material. The classical GP, however, seemed to be not always an indicator of real structure formation. It might also result from pre-gelation and the formation of irregular solid particles in micro-gels.

The two new parameters have proved to be good indicators for the start and the further development of structure formation in low methoxylated pectin gels at varying calcium concentrations. All samples could be evaluated, also those with no clear GP. IST and CST are not seen as complete substitutes of the GP, but they give valuable complementary information and, in case of no clear GP, they are an alternative method for the examination of structure formation. Moreover, the application of the dG′/dt curve allowed the identification of single phases of the gelation, detected by increasing and decreasing structuring velocities. The calculation of structure developing rates, which was made only for the total gelation process, could be applied possibly also to single phases.

ii. The tested LMP gels at pH 3 and with 30% saccharose required at least a small amount of calcium in ionic junction zones for successful gelation; hydrophobic interactions, hydrogen bonds and other mechanisms alone have been proved to be not sufficient, even at the promoting high sugar content. The first phase of the structuring process started at temperatures of ≥ 60 °C by formation of egg-box junction zones and random crosslinks via Ca-bridges. It was supported by hydrophobic interactions and seemed to be nearly completed at about 40 °C. The second and third phase of the structuring process until the end at 10 °C were probably dominated by hydrophilic interactions (assumed below 50 °C) and dimer aggregations (below 25 °C), respectively. The transition of the GP to higher temperature with increasing Ca-content clearly confirmed the importance of the ions for the gelation process. All types of Ca-bridges obviously increased the gelation velocity during cooling considerably and supported the formation of stable elastic gels. Above a certain calcium content, pre-gelation could take place during or immediately after gel preparation that changed the gel structure. These gels were very elastic but became more and more

brittle. The properties of the final gels confirmed the varying gel structures, resulting from different structuring mechanisms in dependence on the calcium content.

iii. The presented interpretations of the rheological parameters and their relation to structuring mechanisms are partly assumptions, yet, and have to be confirmed by further experiments using additional methods and pectin types. The application of the first derivation dG´/dt and the calculated new initial and critical structuring temperatures on the gelation of low methoxylated pectins is, however, a first step into the examination of their general importance for the pectin gelation process.

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