Articles

Influence of Pectin Fine Structure on the Mechanical Properties of Calcium−Pectin and Acid−Pectin Gels

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The in vitro and in vivo functionality of the anionic plant polysaccharide pectin depends not only on the amount of ion-binding groups attached to the polymer but also on the distribution of such groups along the backbone. It has been proposed recently that information regarding this intramolecular distribution can be quantified by defining a degree of blockiness (DB or DB_{abs}), and the usefulness of such measures in discriminating qualitatively between pectins originating from different sources has been demonstrated. Despite this, the value of these parameters in predicting the pseudoequilibrium elastic modulus of gels remains untested. This study seeks to address this problem through the sourcing and in-house modification of a variety of pectins in order to produce a library of distinct representative fine structures. These were subsequently characterized in terms of their relevant properties, including the determination of the proposed DB and DB_{abs}, and the formation of gels of these samples was monitored using small deformation mechanical spectroscopy. In addition to ionotropic calcium gels the effect of the fine structure on acid gelation was also studied.

I. Introduction

Pectin is an acidic polysaccharide that is present in the cell walls of land plants.[1−3] Commercially available pectins are most often extracted from lemon peel or apple pomace.[4] The extracted material consists mainly of a 1,4-linked α-D-galacturonate (typically ∼90%) interspersed by rhamnogalacturonan. Various amounts of neutral sugars can be attached to these regions through rhamnose moieties. The 1,4-linked α-D-galacturonate part of the pectin is referred to as homogalacturonan. Homogalacturonan is a linear and relatively stiff anionic polymer, the C-6 carboxyl group of which can be methyl esterified.[1−3] Pectins are conventionally characterized by this degree of methyl ester content (DM) and divided into high methoxy (HM) pectin with DM > 50% and low methoxy (LM) pectin with DM < 50%. HM pectin is able to form a network in the presence of high sugar concentrations and pH < 4.0[5] whereas LM pectin forms networks in the presence of most di- and trivalent ions, e.g., calcium.[6]

The formation of calcium−pectin networks has been described by the so-called egg box model[7,8] where the calcium is chelated between the carboxyl groups of different pectin chains. For a stable junction zone to be formed a minimum of 10−17 adjacent carboxyl groups is required,[7,9] suggesting that it is not only the DM that plays an important role in the calcium−pectin gelation but also the methyl ester distribution. The methyl ester distribution of pectins can be altered using chemical or enzymic de-esterification of pectins,[1,10,11] and while the reaction mechanism of enzymes in different conditions and from various origin (e.g., tomato and orange) are known,[10,12−14] there is today no common practice on how to best analyze[13−17] the methyl ester distribution and relate it to the viscoelastic properties of pectins. It has been shown using large[1,7] deformation rheological measurements that pectins produced by chemical and enzymic de-esterification processes form calcium−pectin gels with different strengths, but the methyl ester distribution of these pectins were not quantified.

It has recently been proposed that the degree of blockiness (DB)[18,19] and the absolute degree of blockiness (DB_{abs})[20] can be used to quantify the methyl ester distribution of pectins. The DB is calculated from the amount of monomer (1^0), dimer (2^0), and trimer (3^0) of GalA, produced when pectin is incubated with endopolygalacturonase, divided by the amount of free GalA present in the pectin sample, as proposed by Daas and co-workers (eq 1).[18,19]

$$\mathrm{DB} = \frac{[(1 \times 1^0) + (2 \times 2^0) + (3 \times 3^0)]M_w^{\mathrm{GalA}}}{(1 - \mathrm{DM}/100)m_{\mathrm{pectin}}(m_{\mathrm{uronicacid}}/m_{\mathrm{pectin}})} \times 100 \quad (1)$$

Several analytical methods (HPAEC,[17−19] CZE,[21−23] NMR[24]) have been proposed in order to make such measurements.

The DB_{abs} is based on the same idea, but instead of relating the amount of mono-, di-, and trimer liberated by enzymatic

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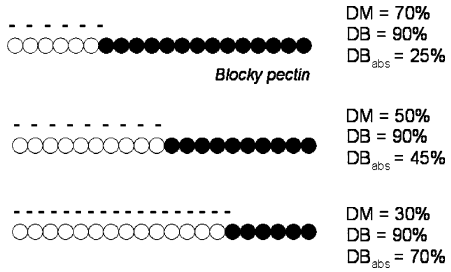

Figure 1. Schematic representation of a blocky pectin and its values of DM, DB, and DB_{abs}. The filled symbols represent the methyl esterified part of the homogalacturonan and the open circles free carboxyl groups (which are of negative charge at neutral pH values).

digestion to the total amount of free GalA residues in the pectin, it is related to the total amount of galacturonate (including methylesterified residues) as defined by Guillotin and co-workers (eq 2).[20]

$$DB_{abs} = \frac{[(1 \times 1^0) + (2 \times 2^0) + (3 \times 3^0)]M_w^{GalA}}{m_{pectin}(m_{uronicacid}/m_{pectin})} \times 100 \quad (2)$$

The difference in the definition of DB and DB_{abs} becomes especially important for extremely blocky pectins as illustrated schematically in Figure 1.

LM pectins with a DM of 30% or below also form networks under acid conditions (pH < 3.0),[25,26] in the absence of sugar. The acid network formation has been suggested to occur after a conformational change from 2_1 to 3_1 helices,[25,27] which gives rise to intermolecular hydrogen bonds between the O(3) and the carboxyl group of adjacent and antiparallel pectin chains and between protonated and unprotonated carboxyl groups of different pectin chains.[28] Thus the methyl ester distribution of pectin should influence the gelation capabilities and rheological properties of acid−pectin gels in addition to calcium−pectin networks.

Although the influence of DB on the gelation of HM pectin in the presence of sugar (at pH 3.0 and 3.5)[29] has been studied, DB and DB_{abs} values of well-characterized samples have not been compared with the small deformation rheological behavior of calcium−pectin or acid−pectin gels. The aim of this paper is therefore to investigate the relative importance of DM, DB, and DB_{abs} on the rheological properties of calcium- and acid-induced pectin networks and to investigate whether it is possible to collect the values of the moduli obtained from gels of different pectin series onto a single master curve using these parameters.

II. Materials and Methods

A. Materials. Two of the three pectin series used in this work were prepared by de-esterification of a commercially available high methoxy pectin (HM6, kindly provided by CP Kelco). One pectin series was de-esterified using alkali (A30, A40, A45, A50, and A60) and a second series (E30, E40, E50, E60, and E70) was de-esterified using a commercial pectin methyl esterase (PME) [EC 3.1.1.11] purchased from Sigma Aldrich (P5400). This PME was extracted from Valencia orange peel and was free from polygalacturonase and pectin acetylesterase activities.[30,31] The de-esterification conditions for the two pectin series have been described in detail elsewhere.[23] The DM and the width of the intermolecular DM distribution of these pectin series were determined using capillary zone electrophoresis.[32,33]

The third pectin series used in this study was kindly provided by CP Kelco [CP37−CP68]. The pectins in this series were de-esterified at 45 °C using a PME extracted from papaya, at a pH of 4.5 and a NaCl concentration of 0.4 M. Additionally, a further commercially available pectin sample with a random intramolecular distribution with a sample averaged DM of ∼35% was also obtained from CP Kelco. The DM values of these pectins were determined by the supplier.

The polygalacturonase [EC 3.2.1.15] that has been used for the digestion of the different pectin series for determination of DB and DB_{abs} was kindly provided by Jacques Benen from the University of Wageningen. It is a pure endoPG II isoform from *Aspergillus niger* prepared as described previously.[34] The preferred substrate for this enzyme is polygalacturonic acid, and it has an absolute requirement for the sugar residues in the active site to be unesterified. The digestion of 0.5% pectin solution was carried out overnight at room temperature. The pectins were dissolved in 50 mM acetate buffer of pH 4.2, and typically 3.4 U (57 nkatals) of endoPG II solution was added to 2.5 mL of pectin solution. The enzyme was inactivated by heating to 85 °C for 10 min.

B. Capillary Zone Electrophoresis. An automated CE system (HP 3D) equipped with a diode array detector was used. Fused silica capillaries with internal diameters of 50 μm and a total length of 56 cm which corresponds to an effective length of 40 cm were used. The detection window consisted of an extended light path of 150 μm. The capillary was thermostated at 25 °C. All capillaries were conditioned by rinsing for 30 min with 1 M NaOH followed by 30 min of 0.5 M NaOH, 15 min of water, and finally 30 min of buffer. Between experiments the capillary was washed for 2 min with 1 M NaOH, for 2 min with 0.5 M NaOH, for 1 min with water, and finally for 2 min with the current buffer. Prior to every run the capillary was rinsed for 2 min with buffer. UV absorbance at 191 nm (with a bandwidth of 2 nm) was used for detection. Samples were loaded hydrodynamically at 5000 Pa with an injection time of 3 to 10 s depending on the concentration of the sample. All experiments were carried out with the inlet anodic and the solutes traveling toward the cathode. The voltage applied during the elution was 15−30 kV depending on the buffer and capillary used. Phosphate buffer at pH 7.0 was used as a CE background electrolyte (BGE) and was prepared by mixing solutions of 0.2 M Na_2HPO_4 and 0.2 M NaH_2PO_4 in a ratio of 1:1.56. The phosphate buffer at pH 7 was subsequently diluted with deionized water to reduce the ionic strength to 90, 50, and 30 mM. All solutions were filtered through 0.2 μm filters (Whatman). Electrophoretic mobilities, μ, are related to the migration times of the injected samples relative to a neutral marker, t and t_0, respectively, by the equation

$$\mu = \mu_{obs} - \mu_{eo} = (lL/V)(1/t - 1/t_0)$$

where L is the total length of the capillary, l is the distance from the inlet to detector, V is the applied voltage, μ_{obs} is the observed mobility, and μ_{eo} is the mobility of the electro-osmotic flow (EOF). Run-to-run variation in the migration times (not electrophoretic mobilities) of species can sometimes be observed owing to the sensitivity of the EOF to the exact state of the capillary wall which can vary with age and conditioning.

C. Rheological Measurements. The viscoelastic properties of the pectin networks were monitored by measuring the storage and loss moduli at a strain of 0.5% and at a frequency of 1 Hz using a Physica UDS 200 (Paar Physica, Stuttgart, Germany). The geometry used was a cup and bob, or serrated cup and bob if required. The pectins were dispersed in deionized water under vigorous stirring. They were heated and held at 60 °C until proper dissolution was obtained (∼30 min). The pH of the pectin solutions was adjusted to pH = 6 with 1 and 0.1 M NaOH. The calcium−pectin gels were formed using controlled calcium release from the $CaCO_3$−glucono-δ-lactone (GDL) system. The $CaCO_3$ and the GDL were quickly dispersed in water (sufficient to dilute the initial 2% pectin solution to 1.5% once the pH was also adjusted) and then immediately added to the pectin solution. The ionic strength was adjusted to 0.1 M by the addition of NaCl, which was added at the same time as the $CaCO_3$ and the GDL. All rheological measurements were carried out at 20 °C. The calcium concentration is

Table 1[a]

pectin	DM/ %	FWHH intermolecular DM distribution	DB/ %	DB_{abs}/ %
E70	69		90	27
E60	60	20	93	36
E50	49	15	91	45
E40	39	10	95	56
E30	30	10	95	67
CP65	65		20	7
CP58	58		21	8
CP48	48		26	13
CP40	40		39	23
CP37	37		57	36
A60	60	5	nd	nd
A50	48	5	25	13
A45	45	6	29	16
A40	37	6	39	18
A35	35	5	40	26
A30	30	4	50	35
LM12	35		36	23

[a] Samples E70−E30 and A30−A60 have been de-esterified from a commercial high methoxy pectin (HM6) using PME extracted from orange (E Series) and alkali (A series). The pectins in the CP series were kindly provided by CP Kelco, and the pectins have been de-esterified using plant PME from papaya. LM12 is a commercial pectin with a random methylester distribution provided by CP Kelco. FWHH is an abbreviation of "full width at half-height", DM for degree of methylation, DB for degree of blockiness, and DB_{abs} for absolute degree of blockiness.

often described relative to the amount of unesterified GalA residues present in the pectin, referred to as R and defined as $R = 2[Ca^{2+}]/[COO^-]$.

D. Rotational Viscometry. The viscosity of the pectin was measured using an AR 1000 from TA instruments. The geometry used was a cone ($\phi = 40$ mm, truncation $= 71$ μm, angle $= 1.59°$) and plate. The viscosity was recorded at $T = 20$ °C and between shear rates of 0.03−1000 s^{-1}.

III. Results and Discussion

A. Characterization of the Methyl Ester Content and Distribution of the Pectins. The three pectin series described in the Materials and Methods section were studied, and the sample average degrees of methyl esterification and the widths of the intermolecular DM distributions were determined by capillary electrophoresis (CE)[23,32,33,36] and are given in Table 1. A full width at half-height (fwhh) is used to parametrize the intermolecular distribution and demonstrates that the intermolecular methyl ester distribution of the E series is broader than that of the A series. This is in agreement with previous studies showing that enzymes that act in a single-chain mechanism (e.g., pPMEs at pH 7.0) will lead to a broad intermolecular methyl ester distribution whereas a multiple-chain, single-attack mechanism (alkaline demethylesterification) gives pectin populations that are more homogeneous with respect to intermolecular charge distribution.[12] Thus, we can assume that the E series have the broadest intermolecular methyl ester distribution followed by the CP series and the A series. Although these differences are clearly detected, we do not expect it to alter the conclusions herein.

The intramolecular methyl ester distributions of the three pectin series were determined by digestion of the pectins using endoPG II as suggested by Daas and co-workers.[18,19] The identification and quantification of the oligomers produced by the endoPG II digestion of the pectins was also carried out using

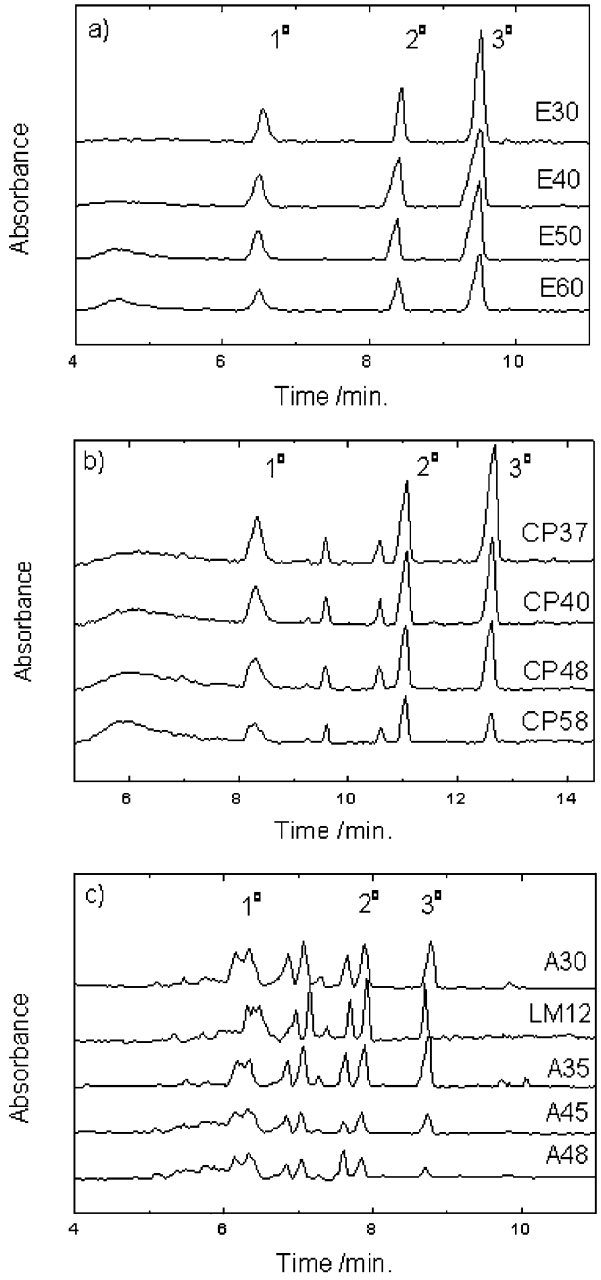

Figure 2. Electropherograms obtained for samples digested with endoPGII (a) E30−E60, (b) CP37−CP58, and (c) A30−A48 and LM12.

CE.[23,35] The electropherograms obtained from the digests of the three pectin series are shown in Figure 2a−c. The digest pattern of the orange PME de-esterified pectins (E series in Figure 2a) is remarkably simple for the pectins with both low and high methyl ester content. The peaks that appear in the electropherograms are those representing the monomer, dimer, and trimer of GalA.

There is an additional peak that elutes for the pectins with a high methyl ester content in the E series (apparent in electropherograms of E50 and E60 in Figure 2a). The early time at which this peak elutes suggests that the peak is related to the presence of larger DP highly methylated pectin that has not been digested by the endoPG II. Above a DP of around 20 it is known that the hydrodynamic friction coefficient and the charge of oligogalacturonides scale symmetrically with molecular length so that it is only the average charge per residue that determines the position of elution.[21,35] This means that highly methyl

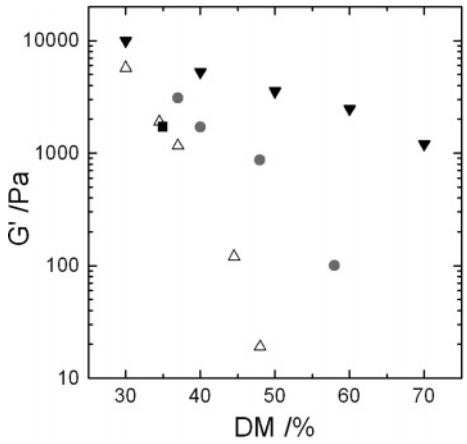

Figure 3. *G′* after 700 min as a function of DM for alkali (triangles), papaya PME (gray circles), orange PME (black down triangles) de-esterified pectins, and one commercial pectin sample, LM12 (black square) (polymer concentration = 1.5% and pH = 6). The final calcium concentration corresponds to an *R* value of 0.3.

esterified fragments migrate faster than the more highly charged oligomers despite their size.

Figure 2b shows the electropherograms for the enzymically de-esterified pectin series obtained from CP Kelco. As observed for the E series, the types of oligomers released do not change as DM increases. The same oligomers are present as the DM values increase from 37% to 68%. The additional peak that was previously suggested to indicate the presence of high molecular weight and high methyl esterified pectin appears as DM increases. Compared to the E pectin series, there are some additional oligomer peaks present (most probably 5^1 and 4^1), i.e., respectively a pentamer and a tetramer of GalA with one methyl ester group attached,[22,36] suggesting that the parts of the pectin that are accessible to the endoPG II contain more methyl ester groups than does the E series. In the generation of these samples the p-PME was used away from its pH optimum (M. E. Hansen, personal communication), which is likely to lead to a less processive action pattern (as discussed above).

The electropherogram obtained for the alkali de-esterified pectins (A series) shows a different pattern as seen in Figure 2c. A larger variety of oligomers is produced among which many are methyl esterified. The majority of peaks for the low DM pectin (A30) can be identified,[22,23,35] whereas as DM increases, the electropherograms get more complex and not all peaks can be resolved or identified. The early elution times of many of the additional peaks indicate that they correspond to esterified oligomers with low charge. Thus, as DM increases for the alkali de-esterified pectins, the blocks of consecutive GalA residues decrease in length, resulting in progressively more and more esterified oligomers. This is in contrast to the two pPME de-esterified pectins, which irrespective of DM appear to contain blocks of consecutive GalA residues containing little or no esterified groups. As discussed, this is consistent with current models of the action of pPMEs.[30,31]

Having constructed a sample set of well-characterized pectins, the influence of differences in the methyl ester distribution between the three pectin series on the rheological behavior of calcium− and acid−pectin networks will be discussed in the rest of the paper.

B. Influence of the Methyl Ester Content and Distribution on the Rheological Properties of Calcium−Pectin Networks. The modulus of the calcium−pectin networks obtained using the three different pectin series for a fixed *R* value (0.3) are shown in Figure 3. Initial studies showed that the pectins from the E series were more calcium sensitive than the pectins from the CP and the A series, leading to turbid gels with syneresis at *R* values approaching 0.5.[6] This is in agreement with previous studies demonstrating that pPME de-esterified pectins bind calcium more strongly than alkali de-esterified pectins.[37] Therefore, $R = 0.3$ was selected so that all samples formed gels that were transparent and showed no sign of syneresis. The gels were formed using a controlled release of calcium, and the moduli are measured after 700 min, i.e., when all the calcium is released and any significant evolution of the gel has ceased. It is seen (in Figure 3) that the pectins de-esterified using pPME (E and CP Kelco series) form stronger calcium gels for all DM values studied and that they are able to form calcium−pectin gels at higher DM values than the alkali de-esterified pectins. The threshold for calcium gelation (at $R = 0.3$) for the alkali de-esterified pectin is at DM $\sim$ 49%, which is in agreement with the commonly quoted DM threshold for pectin networks governed by electrostatic interactions (DM $<$ 50%) compared to those governed by hydrogen bonding and hydrophobic interactions (DM $>$ 50%).[5] The DM thresholds for calcium gelation of the pPME de-esterified pectins (E series and CP Kelco series) are at ~70% and ~60%, respectively. This clearly shows that simple knowledge of the methyl ester content of a pectin sample is not sufficient to predict the ability of the polymer to form a gel or the properties of the network, in agreement with other studies.[7,9,23] It can also be seen in Figure 3 that *G′* increases as DM decreases for all three pectin series; that is, within each series with fixed *R* value and concentration a relationship with DM is observed. However, there does not seem to be a simple relation to predict the increase in the storage modulus for pectins of similar DM but produced using different methods (different pPME enzymes and alkali de-esterification). It is known from the digestion pattern of the three pectin series that the intramolecular methyl ester distributions of the pectins within the three pectin series are different. It was therefore tested whether a more universal prediction of the storage modulus for calcium gels of pectins with various DM and from different conditions of de-esterification could be obtained by relating *G′* to the intramolecular methyl ester distribution instead of simply the methyl ester content. It has been suggested that this distribution can be described by both the degree of blockiness (DB)[19] and the absolute degree of blockiness (DB_{abs})[20] as described in the Introduction. It should be noted that the definition of DB and DB_{abs} is not strictly dependent on the origin of the degrading enzyme. It is possible that the acceptance for methyl ester groups within the active site or adjacent to it differs for endo PGs from different origins. This would lead to different values of DB and DB_{abs} between research groups using PGs of different origin (Dr H. Schols, personal communication); therefore, it is the difference in the DB values between substrates, for the same enzyme, that should be compared.

Figure 4a shows that there is no simple correlation between the storage modulus of calcium−pectin gels and DB. This is not unexpected as the values of DB do not change significantly as DM changes since the ratio of the amount of mono-, di-, and trimer are based on the free GalA in the pectin and not the total amount of GalA. DB_{abs} takes the total amount of GalA into account, and plotting the storage modulus of the calcium−pectin gels as a function of DB_{abs} makes the data collapse better onto a single master curve (Figure 4b) than plotting the data as a function of DB (Figure 4a). As the calcium ratio *R* was kept at 0.3, the absolute calcium concentration between the different pectin gels was also different depending on the DM of the pectins. In an attempt to compensate for this, the modulus was

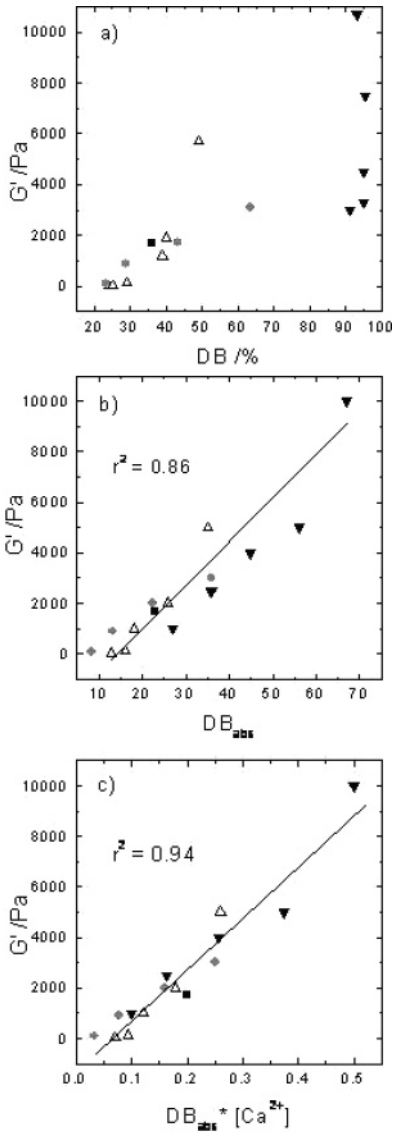

Figure 4. *G′* as a function of (a) DB, (b) DB_{abs}, and (c) $DB_{abs}[Ca^{2+}]$ for the A series (open triangles), the CP Kelco series (gray circles), the E series (black down triangles), and LM12 (black square). The polymer concentration = 1.5%, pH = 6, and the final calcium concentration corresponds to an *R* value of 0.3. *G′* is taken after 700 min.

plotted against $DB_{abs}[Ca^{2+}]$, and this slightly improves the mapping of the data onto a single relationship, as indicated in Figure 4c. These results demonstrate that the strength of the calcium−pectin gels correlates better with their DB_{abs} values than with DM or DB. DB is a good indicator of calcium sensitivity, but since it is based on the free galacturonate content and not the total galacturonate content, pectins with similar blockiness but different DM give similar values for DB. The correlation between the strength of the calcium−pectin gels and DB_{abs} was improved by taking the total calcium concentration into account. Studying systems in which the *R* value could be changed in order to observe the effects of larger variations in calcium concentration forms part of ongoing work.

C. Influence of the Methyl Ester Content and Distribution on the Rheological Properties of Acid−Pectin Networks. It was shown above that the methyl ester distribution of the pectin strongly influences the ability of the pectin to form calcium−pectin networks and the strength of these networks. This can be rationalized from the fact that 10−17 adjacently bound calcium ions are needed to form a stable junction zone.[7] This

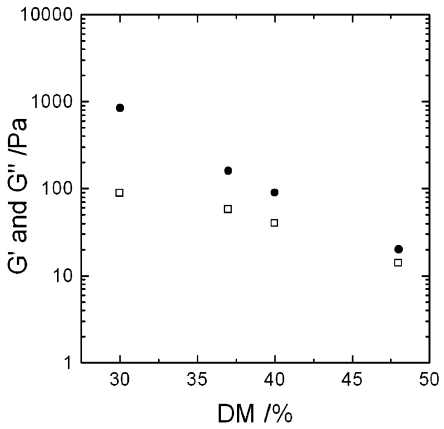

Figure 5. Influence of DM on *G′* and *G″* (measured at a strain of 0.5% and a frequency of 1 Hz) on acid−pectin networks (polymer concentration = 1.5%, pH = 3.0, and $T = 5$ °C). The solid symbols represent *G′* and the open symbols represent *G″*. The pectins used are E30, CP37, CP40, and CP48.

section reports on the possible influence of the methyl ester distribution on acid−pectin networks. The influence of the total methyl ester content of the pectins (E30, CP37, CP40, and CP48) on their ability to form acid−pectin networks is shown in Figure 5. The modulus of the network increases as DM decreases, i.e., the carboxyl group content of the pectin samples increases. This is in accordance with the proposed model of acid−pectin networks where the network is governed by hydrogen bonds between the unprotonated and protonated carboxyl groups of pectin chains.[25,28] Also the melting and gelation temperatures of the pectin networks increase as DM decreases, suggesting that there is a larger free energy change involved in the network formation as DM decreases. A more detailed report on the melting behavior will be published elsewhere.

The acid gelation of three pectin samples (CP37, A35, and LM12), all with approximately the same methyl ester content (DM ∼ 35%) but with different intramolecular methyl ester distributions, was investigated in order to study the influence of the distribution on the ability of pectins to form acid networks, in analogy to the study on calcium−pectin networks described above. These three pectin samples were selected based on having a DM at which all structures had a chance to gel (only pectins that had been de-esterified using pPME gelled at DM values that were >35%).

The electropherograms of both LM12 and A35 (Figure 2c) contain several peaks representing methyl esterified oligomers whereas the electropherogram obtained from the endoPG II digested CP37 (Figure 2b) is simpler with peaks corresponding predominantly to unesterified oligomers. This suggests, as discussed above, that the LM12 and A35 contain shorter blocks of adjacent GalA units or longer blocks with mixed content but long enough for the enzyme to cleave them into short blocks containing methyl ester groups. This is also reflected in the values of DB, which were calculated to be 60% for CP37, 40% for A35, and 36% for the LM12 and of DB_{abs} which were 36%, 26%, and 23%, respectively.

Figure 6 shows that the most blocky sample in this group, CP37, forms acid−pectin gels at pH values of ≤3.5 (which was the highest pH value studied). The A35 sample forms a weak acid gel but only for the pH value of 2.0, whereas the least blocky pectin (LM12) does not form an acid gel at any of the pH values studied (2.0−3.5). Even systems with higher concentrations of LM12 (2.5%) did not form an acid gel. This demonstrates that the intramolecular methyl ester distribution plays a more important role for the acid−pectin gelation

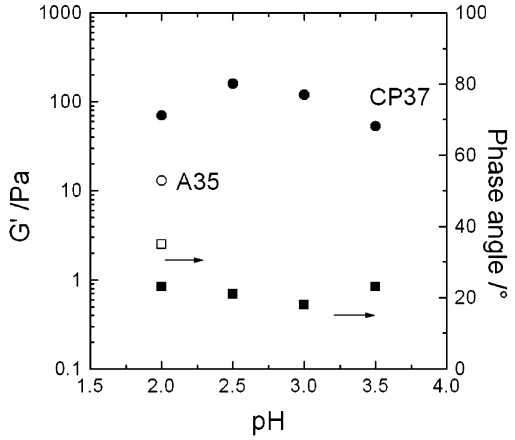

Figure 6. Influence of pH on G' (circles), and the phase angle (squares) (at a strain of 0.5% and frequency of 1 Hz) for CP37 (solid symbols) and A35 (open symbols). Polymer concentration = 1.5% and $T = 5$ °C.

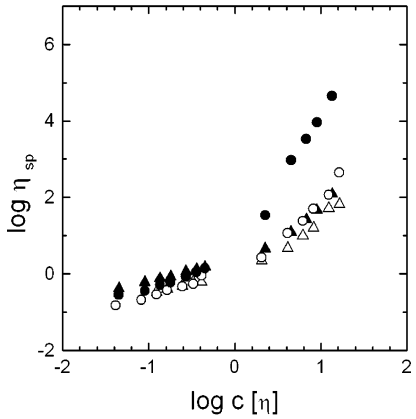

Figure 7. Zero shear viscosity as a function of polymer concentration at pH = 3 (solid symbols) and pH = 5 (open symbols) for CP37 (circles) and LM12 (triangles) at $T = 20$ °C.

compared to the total methyl ester content and suggests that co-operativity is important for the formation of junction zones in acid−pectin gels. Similar results have been reported for acid alginate gels.[38] The intermolecular methyl ester distribution may also have an impact on the acid gels, and a more detailed study of this aspect will be carried out in the future. However, it can be assumed that the intermolecular methyl ester distribution of the CP series and the A series differ less than that for the E series and the A series that were compared in Part B, and we do not expect the conclusions expounded here to be affected by the intermolecular distribution.

The different rheological behavior of the blockiest (CP37) and least blocky pectin structures (LM12) of similar DM (~35%) were further studied using rotational viscometry. It should be noted that while the molecular weight of these samples has not been explicitly measured, the intrinsic viscosities were very similar, implying that they are not significantly different. It is known that the variation in viscosity as a function of polymer concentration for many polysaccharides (and indeed polymers in general) follows a master curve if the log zero shear specific viscosity (log η_{sp}) of the solution is plotted as a function of the log space occupancy of the polymer coils (log $c[\eta]$).[39] In the simplest coil overlap model it is predicted that the increase in log η_{sp} as a function of log $c[\eta]$ will occur in two linear regimes[39] which is indeed found to be the case for the LM12 and CP37 solutions as shown in Figure 7. The critical polymer concentration where the two lines cross is commonly referred to as c^*.[39]

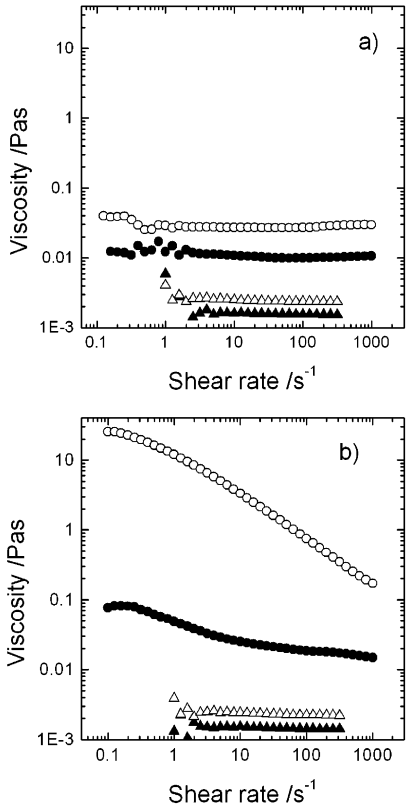

Figure 8. Viscosity as a function of shear rate for 0.1% (triangles) and 1.5% (circles) solutions of CP37 (open symbols) and LM12 (solid symbols) at a pH of (a) 5.0 and (b) 3.0.

It can be seen in Figure 7 that the slopes for log viscosity as a function of log reduced polymer concentration are similar for LM12 and CP37 when they are in the dilute regime and also independent of pH (for pH 3 or 5). However, as pH is decreased to 3 and the solution enters the entangled regime, the increase of viscosity as a function of the reduced pectin concentration is greater for the pectin solution containing the blocky pectin (CP37) than for the pectin solution with the least blocky pectin (LM12). Similar discrepancies from the "master curve" have been shown for polysaccharides that are believed to form interactions in solutions in addition to physical entanglement of overlapping coils.[39−41] The extent of specific chain−chain associations of this type would be expected to increase with increasing concentration, thus in effect raising the average molecular weight of the polymer and hence the viscosity.[39] Thus, the results presented suggest that pectin with a more blocky architecture indeed has a greater tendency to form chain−chain associations, increasing the M_w of loosely attached pectin "bundles". Since this behavior is only observed at low pH (3.0) it is possible that hydrogen bonds are the forces keeping the pectin "bundles" together. This is also reflected in the shear thinning behavior of the pectin solutions (Figure 8) which clearly differs depending on the intramolecular methyl ester distribution of the dissolved pectin.

In Figure 8a the viscosities of solutions of random (LM12) or blocky (CP37) pectin show little shear rate dependence at pH 5. However, the viscosities of the semidilute solutions increase as pH decreases to 3 (Figure 8b) for both polymers, although the increase of viscosity of the CP37 solution is considerably larger than for the LM12. Furthermore, the shear rate dependence of the viscosity of the CP37 solution is large

compared to the LM12 solution at pH 3. The subsequent decrease in viscosity with increasing shear may be attributed to the progressive breakdown of an intermolecular network,[40] again suggesting that the blocky pectin is more prone to intermolecular aggregation at $c > c^*$ and low pH values where hydrogen bonds may promote aggregation. Studies by another group[42] have also shown that the increase in the reduced viscosity as a function of pectin concentration is different for LM and HM pectin where the LM pectin had been de-esterified using orange PME and suggest that the driving force for aggregation of the orange PME de-esterified LM pectin is coming at least in part from hydrogen bonding.[42]

IV. Conclusions

The small deformation rheological response of calcium−pectin gels correlates reasonably well with the values of the recently proposed DB_{abs} (particularly the product $DB_{abs}[Ca^{2+}]$), irrespective of the polymeric fine structure. In addition, the intramolecular methyl ester distribution also influences the capabilities of LM pectins to form acid gels. LM pectins of high blockiness are able to form acid−pectin gels at higher DM values and at higher pH values compared to pectins of low blockiness.

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References and Notes

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