Relation between Rheological Properties of Pectin Gels and Pectin Fine Structure

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ABSTRACT

This study investigates the influence of pectin fine structure (i.e. methyl ester content and distribution) on the rheological properties of calcium induced pectin networks. The methyl ester distribution was assessed using the definition of the degree of blockiness (DB) and absolute degree of blockiness (DB_{abs}). The study showed that 1) the ability to form calcium induced pectin networks depends more on the DB and DB_{abs} than the degree of methylation (DM) and 2) that the absolute value of gel strength is increased for pectins with a high degree of blockiness. These results were explained by the effectiveness of the pectins to bind calcium. A pectin with a blocky methyl ester distribution will bind the available calcium in stable junction zones compared to a pectin with random distribution of methyl esters where some calcium will be less effectively bound. It is concluded that the methyl ester distribution is crucial for predictions of the rheological properties of calcium pectin networks and that DB_{abs} seems to be a valid route to assess the methyl ester distribution and predict its rheological properties.

INTRODUCTION

Pectin is an acidic polysaccharide that is present in the cell wall of plants[1]. It is commercially extracted mainly from lemon peel and apple pomace[2]. The extracted material consists of a 1,4 linked α-D-galacturonate (typically ~90%) to which neutral sugars are attached (< 10%). This part of the pectin is referred to as homogalacturonan.

Homogalacturonan is a linear and relatively stiff anionic polymer[3], which may be esterified by methyl groups on the C-6. Pectins (or homogalacturonan) are typically characterised by their degree of methylation (DM) and the network formation capabilities of pectins are often related to this value. High methoxy pectins (DM>50%) form networks under acidic conditions and at high solid content whereas low methoxy pectins (DM<50%) form ion induced networks.

Calcium induced pectin networks are formed according to the egg box model[4]. This model suggests that gelation will proceed in two steps where the first step is a rapid formation of dimeric junction zones where calcium is chelated between the negatively charged carboxyl groups of two pectin chains. It is believed that the

formation of a stable junction zones requires a block of approximately 12-16 consecutive free galacturonic acid (GalA) units[4,5]. Thus, the methyl ester distribution will also influence the gelation capabilities and the rheological properties of calcium pectin networks. The degree of blockiness (DB)[6,7] and the absolute degree of blockiness (DB_{abs})[8] have been proposed as two alternative parameters describing the methyl ester distribution of pectins.

The second step in the egg-box model relates to the amount of calcium present during gelation of pectin. At low calcium concentrations only dimeric junction zones are formed whereas at higher calcium concentrations larger aggregates of dimeric junctions can form, which may lead to syneresis and shrinkage of the gel.

The aim of this study is to investigate, which parameter, (DM, DB or DB_{abs}) that describes the capabilities of pectin to form ion induced networks and predicts the gel strength most accurately. Furthermore, we investigate the impact on gel strength as a function of calcium concentration using pectin with different (intramolecular) methyl ester distribution.

MATERIAL & METHODS

Materials

Two of the three pectin series used in this work were prepared by esterification followed by de-esterification of a commercially available high methoxy pectin (DM=72%, that was esterified to DM=81%). One pectin series was de-esterified using alkali (A30-A60) and a second series (E30-E70) was de-esterified using a commercial pectin methyl esterase (PME) [EC 3.1.1.11] purchased from Sigma Aldrich (P5400). The de-esterification conditions for the two pectin series have been described elsewhere[9]. The DM of these pectin series were determined using capillary zone electrophoresis[9] and are presented in Table 1.

The third pectin series used in this study was kindly provided by CP Kelco [CP37-CP68]. The pectins in this series have been de-esterified by the supplier using PME from papaya. The DM values of these pectins were determined by the supplier (Table 1).

Calculation of DB and DB_{abs}

The degree of blockiness (DB) is calculated from the amount of monomer (1^0), dimer (2^0) and trimer (3^0) of GalA produced as pectin is incubated with endopolygalacturonase (endoPG), divided by the amount of free GalA present in the pectin sample, as proposed by Daas and co-workers[6,7] (Eq. 1).

$$DB = \frac{\left(1\times 1^0 + 2\times 2^0 + 3\times 3^0\right)\times M_w^{GalA}}{\left(1 - DM/100\right)\times m_{pectin}\times\left(m_{uronicacid}/m_{pectin}\right)}\times 100 \qquad (1)$$

The absolute degree of blockiness (DB_{abs}) is based on the same idea but instead of relating the amount of mono-, di-, and trimer liberated by enzymatic 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[8] (Eq. 2)

$$DBabs = \frac{\left(1\times 1^0 + 2\times 2^0 + 3\times 3^0\right)\times M_w^{GalA}}{m_{pectin}\times\left(m_{uronicacid}/m_{pectin}\right)}\times 100 \qquad (2)$$

The endoPG digestion and determination of digestion products were performed as described previously[9,10,11]. The values obtained for DB and DB_{abs} are presented in Table 1.

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

Table 1. Values of DM, DB and DBabs of the pectin used in this study, where 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. This series has been de-esterified using plant PME from papaya.

Pectin	DM /%	DB /%	DB_{abs} /%
E70	69	90	27
E60	60	93	36
E50	49	91	45
E40	39	95	56
E30	30	95	67
CP65	65	20	7
CP58	58	21	8
CP48	48	26	13
CP40	40	39	23
CP37	37	57	36
A60	60	n.d.	n.d.
A50	48	25	13
A45	47	24	13
A45a	45	29	16
A40	37	39	18
A35	35	40	26
A30	30	50	35

serrated cup and bob. The pectins were dispersed in deionised water under vigorous stirring. They were heated and held at 60°C until proper dissolution was obtained (~ 30 minutes). 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 delta 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 adjusted) and 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 described relative to the amount of unesterified GalA residues present in the pectin, and referred to as R. R is defined as $R = 2[Ca^{2+}]/[COO^-]$.

RESULTS AND DISCUSSION

Correlation between calcium pectin gel strength and pectin fine structure

The modulus of the calcium pectin networks obtained using three different pectin series at a fixed R value (0.3) are shown in Figure 1.

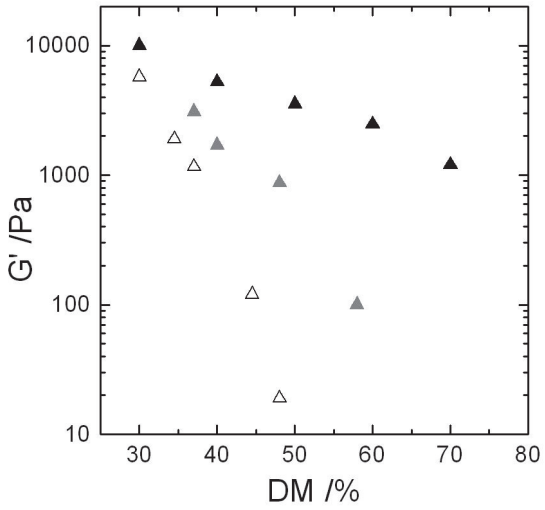

Figure 1. G' after 700 minutes as a function of DM for alkali (open symbols), papaya PME (grey symbols) and orange PME (black symbols) de-esterified pectins (polymer concentration = 1.5% and pH = 6). The final calcium concentration corresponds to an R value of 0.3.

These 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 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 ~49%, which is in agreement with the commonly quoted DM threshold between pectin networks governed by electrostatic interactions (DM<50%) and those governed by hydrogen bonding and hydrophobic interactions (DM>50%)[14]. 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 shows that 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, which is in agreement with other studies[4,11,13].

Figure 1 also shows that G' increases as DM decreases for all three pectin series. However, there does not seem to be a simple relation to predict the increase in the storage modulus for pectin of similar DM but produced using different methods (different pPME enzymes and alkali de-esterification).

It was therefore tested whether a better 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 expressed as DB or DB_{abs} instead of the methyl ester content (DM).
The does not appear to be a simple relation between the modulus of calcium pectin gel with an R value of 0.3 and different DB (Figure 2). This could potentially be explained by the fact that DB only takes the free galacturonic acid content into account and not the total pectin chain. The difference in the definition of DB and DB_{abs} becomes especially important for extremely blocky pectins as these will end up having similar DB despite having different DM.

Figure 4 shows the modulus of the pectin series as a function of DB_{abs}. It is seen that plotting the storage modulus of the

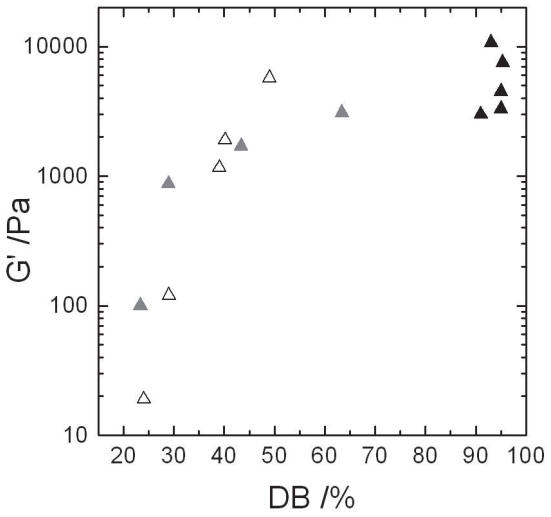

Figure 2. G' as a function of DB for the A series (open symbols), the CP Kelco series (grey symbols) and the E series (black symbols). The polymer concentration = 1.5%, pH = 6 and the final calcium concentration corresponds to an R value of 0.3. G' is taken after 700 minutes.

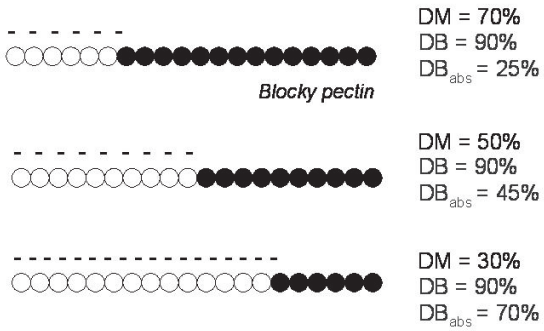

Figure 3. Schematic representation of a blocky pectin and its values of DM, DB and DB_{abs}.

calcium pectin gels as a function of DB_{abs} makes the data collapse better onto a single mastercurve.

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 plotted against $DB_{abs} \times [Ca^{2+}]$ and this

slightly improves the mapping of the data onto a single relationship, as shown in Figure 5.

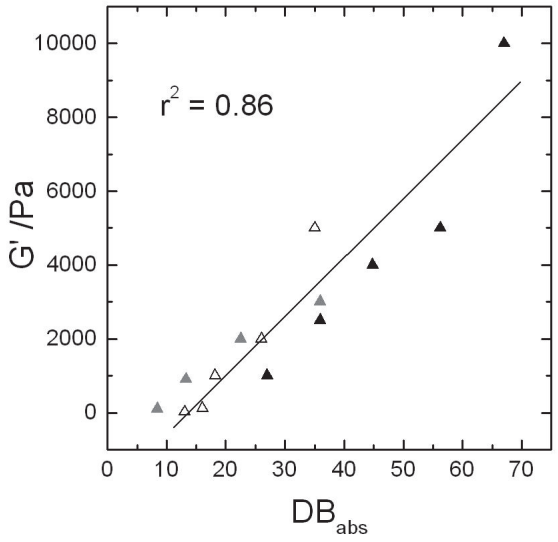

Figure 4. G' as a function of DB_{abs} for the A series (open symbols), the CP Kelco series (grey symbols) and the E series (black symbols).

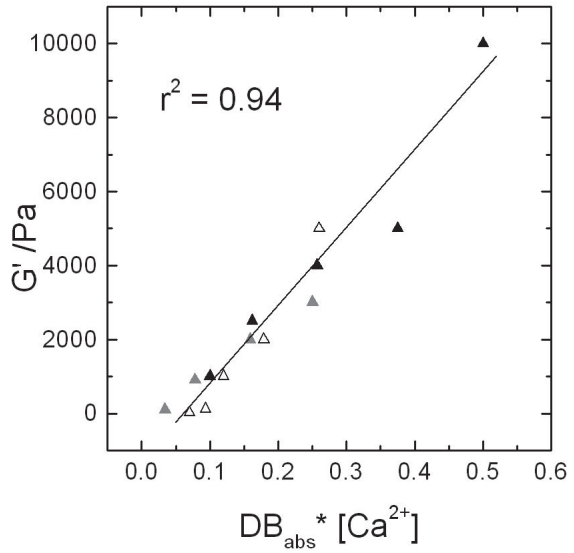

Figure 5. G' as a function of DB_{abs}*[Ca] for the A series (open symbols), the CP Kelco series (grey symbols) and the E series (black symbols).

Correlation between calcium binding and pectin fine structure

The effect of calcium concentration on pectin gel strength was studied on the A and E series as these two series differ the most in charge distribution. Figure 6 shows the increase in G´ for A45 and E50, two pectins with large difference in DB_{abs} (13 and 45, respectively) but with similar DM (47 and 49%). G´ increases steeply for both the alkali and the enzymically de-esterified pectin as the R value goes from 0-0.5. For R values above 0.5 it becomes difficult to measure the viscoelastic properties of E50, probably due to slippage (despite the use of serrated cup and bob). Slippage might be the result of extensive aggregation in the network, which would lead to a decreased water-holding capacity. However, it is important to point out that neither opacity nor syneresis were observed on visual inspection of the gels. That the threshold value between homogeneity and possible aggregation and inhomogeneity is observed to be at R = 0.5 agrees well with the egg box model[4]. An R value of 0.5 corresponds to half the stoichiometric ratio of calcium to free GalA groups, and it is at this R value that the largest extent of dimeric junction zones should be formed. Above this R value (R > 0.5), the calcium concentration is sufficient to induce dimer-dimer aggregation or dimer-dimer "sheets", leading to extensive aggregates and a decreased water-holding capacity. Indeed, it has been reported that high calcium concentrations, in a pH range of 3 to 7, can be detrimental to the network by increasing the crosslinks to such an extent that pectin is precipitated[4].

A different behaviour to that of the enzymatically de-esterified pectin was observed for the alkali de-esterified pectin of DM 45%. G´ reached a maximum value at R ~ 0.9 which is close to the stoichiometric equivalence (R = 1.0) of carboxylate and calcium in the system. The fact that the modulus increased up to a stoichiometric amount of calcium confirms

that not all calcium is bound into active and stable junction zones. That is, calcium is bound to too short GalA blocks to actively contribute to the network. G´ reaches a plateau value as the calcium concentration increases to R values > 0.9.

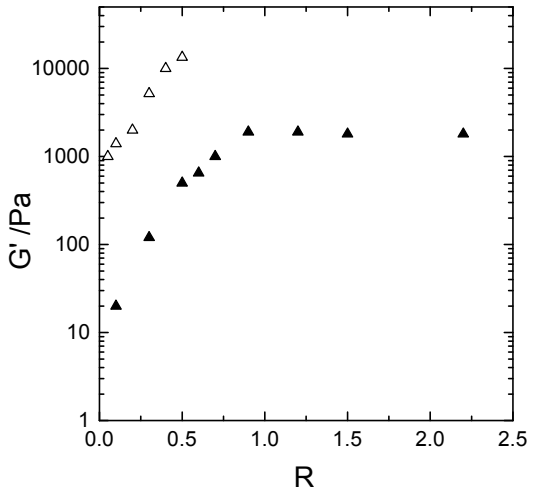

Figure 6. G´ as a function of R for two pectin samples of DM close to 45 %, produced by de-esterification with alkali (filled symbols) and enzymes (open symbols). The G´ value is taken after 700 minutes.

The enzymically de-esterified pectin with a high DB formed a network for a calcium concentration corresponding to an R value of 0.05 whereas an R values of 0.1 was required to initiate network formation of the alkali de-esterified pectin with low DB. The values of phase angle for the different gels show a dominant elastic behaviour for all gels. However, increasing R value from 0.2 results in an increase of phase angle (from 1 to 2 degrees) for the blocky pectin whereas the phase angle for the pectin with low blockiness is stable until an R value of 1.5 (stable at 1 degree).

The fact that a blocky pectin forms a calcium induced network at lower R values and that extensive aggregation seems to occur at lower R values than for a less blocky (alkali de-esterified) pectin of the same DM suggests that the blocky pectin chelates calcium more efficiently into stable and load bearing junction zones.

Figure 7 shows further the capacity of binding calcium ions in load bearing junctions depending on the pectin fine structure. The filled triangles in Figure 7 represent the increase of G´ as a function of calcium concentration for E50 and for the E series of DM from 30 to 70%. It is seen that the two series of triangles nearly superimpose although the filled triangles are all of DM 45% and the open triangles of DM 70, 60, 50, 40 and 30%, thus suggesting that G´ is independent of DE for the enzymically de-esterified pectins of high DB (the DB values are in this case between 90 and 95%) but depends on the calcium concentration.

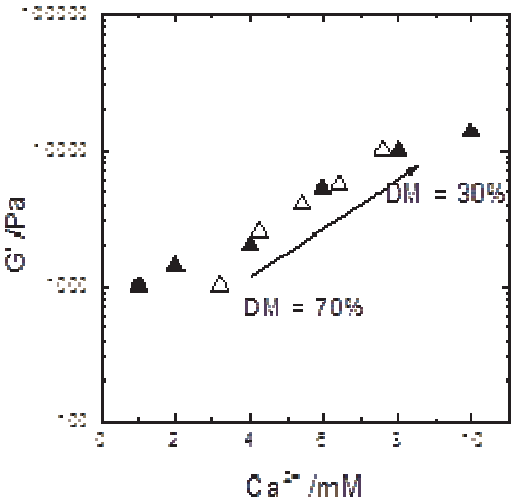

Figure 7. Comparison of the effects of the increase in G' as a results of the calcium concentration and DM of the pectin samples with high degree of blockiness. Filled triangles represent G' as a function of calcium concentration for a pectin of DM ~45%. Open triangles represent G' as a function of both calcium concentration and DM in the range of 70 < DM <30%. DM decreases for the unfilled triangles in the direction of the arrow.

This finding indicates that the limiting parameter for forming junction zones for

pectins of high DB is the bridging calcium ions and not the probability of having a certain amount of consecutive GalA residues capable of forming a stable junction zone, nor the relative ratio between methyl-esterified and unesterified GalA residues.

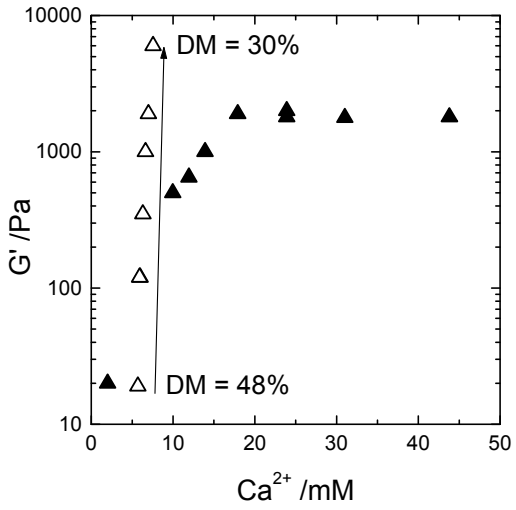

Figure 8. Comparison of the effects of the increase in G' as a results of the calcium concentration and DM of the pectin samples with varying degree of blockiness. Filled triangles represent G' as a function of calcium concentration for a pectin of DM ~45%. Open triangles represent G' as a function of both calcium concentration and DM in the range of 48 < DM <30%. DM decreases for the unfilled triangles in the direction of the arrow.

Figure 8 shows the same procedure for the alkali de-esterified pectins. G´ is plotted as a function of calcium concentration for the pectin of DM 45% (filled triangles) and for the pectins of 30% < DM < 49% (open triangles). In this case, the two series do not superimpose. Instead, G´ increases steeply for the series of open triangles, which represents the alkali de-esterified pectins with various DM values. For instance, the pectin of DM 30% has a G´ value almost an order of magnitude higher than the pectin of DM ~45% at approximately the same calcium concentration (~10 mM). It is in this case difficult to tell whether G´ is mainly influenced by DB or DM for the alkali de-esterified pectins since DB and DM are closely coupled for these pectins.

It has been shown that the intramolecular distribution of methyl ester groups is an important factor to account for when predicting ability of pectin to form calcium gels and the strength of those. This study showed that DB_{abs} is better to use than DB. However, it should be noted that endoPGs of different origin may differ in their acceptance for methyl ester groups within the active site or adjacent to it (Dr H. Schols, personal communication), which consequently will give different value of DB and DB_{abs}, making comparisons between scientific groups and commercial partners difficult unless the endoPG of the same origin is used in all studies. pectins difficult.

CONCLUSIONS

The rheological characterisation of these pectins showed that pectin with a high degree of blockiness chelates the calcium ions in stable junction zones more effectively than pectins with lower degree of blockiness. Pectin with a high degree of blockiness will therefore be able to form calcium gels at a higher degree of methylation, lower calcium concentration and give stronger gels compared to pectins with less blocky charge distribution. However, they may also show a higher tendency for network collapse due to too high calcium concentrations. Different routes to determine the degree of blockiness have been proposed, DB and DB_{abs}, this study suggest that DB_{abs} predicts gelation and gel strength for different pectins (alkali and enzymically de-esterified) better than DB and DM.

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