Effect of de-(methyl)esterification on network development and nature of Ca2+-pectin gels: towards understanding structure-function relations of pectin

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Effect of de-methylesterification on network development and nature of Ca\(^{2+}\)-pectin gels: Toward understanding structure-function relations of pectin

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Abstract

Pectins of varying degree and pattern of methylesterification were produced through controlled de-esterification of highly esterified citrus pectin, using carrot pectin methyl esterase (PME) (P-pectins), *Aspergillus aculeatus* PME (F-pectins) or sodium hydroxide saponification (C-pectins). Estimation of the degree of methylesterification (DM) and quantification of the pattern of methylester distribution in terms of absolute degree of blockiness (DB\textsubscript{abs}) enabled the characterisation of pectins. Characterized pectins were used for the preparation of Ca\textsuperscript{2+}-pectin gels with varying calcium ion (Ca\textsuperscript{2+}) concentration. The rheological characteristics of produced gels were evaluated by means of small-amplitude oscillatory tests. During gel formation, gel strength was monitored so as to allow assessment of network development. Based on the evaluation of mechanical spectra, the nature of the cured gels was established. Depending on Ca\textsuperscript{2+} concentration as well as DM and DB\textsubscript{abs}, gels prepared from specific C-pectins (48 \(\geq\) DM \(\geq\) 26%; 9 \(\leq\) DB\textsubscript{abs} \(\leq\) 37%) and F-pectins (64 \(\geq\) DM \(\geq\) 29%; 9 \(\leq\) DB\textsubscript{abs} \(\leq\) 50%) showed a striking decrease of the gel strength with time, while gel networks produced from other pectins either displayed a continuous increase of the gel strength or verged toward pseudo-equilibrium within the observation time of 5 h. Furthermore, the DM, DB\textsubscript{abs} and Ca\textsuperscript{2+} concentration influenced the evolution of Ca\textsuperscript{2+}-pectin networks from “structured liquids” to “strong gels”. Based on the experimental results, specific mechanisms of Ca\textsuperscript{2+} interactions with pectins were (also) considered.

**Keywords:** Pectin - De-esterification - Absolute degree of blockiness - Ca\textsuperscript{2+}-pectin gels - Rheology - “Egg-box” model – Pectin gels classification
List of abbreviations

$\text{DB}_{\text{abs}}$ = absolute degree of blockiness

$\text{DM}$ = degree of methylesterification

$\text{GalA}$ = galacturonic acid

$\text{HMP}$ = high methylesterified pectin

$\text{LMP}$ = low methylesterified pectin

$\text{LVE}$ = linear viscoelastic

$\text{NM-GalA}$ = non-methylesterified galacturonic acid

$\text{NM-MDT-GalA}$ = non-methylesterified mono-, di- and tri-galacturonic acid

$\text{PDP}$ = partially de-esterified pectin

$\text{PM}$ = pattern of methylesterification

$\text{PME}$ = pectin methylesterase

$\text{PG}$ = polygalacturonase
1. Introduction

Pectin is a complex polysaccharide abundantly found in the middle lamella of fruit and vegetable tissues. Generally, this macromolecule consists of homogalacturonan (HG) and rhamnogalacturonan I & II domains. Homogalacturonan consists of a linear homopolymer of $\alpha(1-4)$ linked -D- galacturonic acid which, naturally, can be methylesterified at C-6 carboxyl and/or acetylated (Vincken, Schols, Oomen, McCann, Ulvskov, Voragen & Visser, 2003). Owing to the structural features of HG, pectin is used as “textural” polymer in numerous applications. The extent of methyl esterification (DM) has been considered as a key parameter defining the gelling properties of the polymer. This functional property of pectin enables the use of isolated commercial pectin as gelling, thickening or stabilizing agent in food systems. Generally, high methylesterified pectins (HMPs) with DM $\geq$ 50% can form gels in the presence of soluble solids such as sugars (Oakenful, 1991; Thibault & Ralet, 2003), whereas low methyl esterified pectins (LMPs) of DM < 50%, are associated with gelation by divalent cations (Thibault & Ralet, 2003; Endress, Mattes & Norz, 2006). Soluble solids induced gelation (for HMPs) requires low pH (COOH groups) while gelation in the presence of divalent cations (specifically Ca$^{2+}$ in food applications) takes place under high pH (COO$^{-}$ groups) conditions. The latter gelation technique has been largely used in the production of low-calorie healthy foods (Endress et al., 2006). In addition, texture preservation in some processed fruits and vegetables has been related to the formation of in planta Ca$^{2+}$-pectin gel networks, which hinder pectin depolymerisation and solubilisation (Sila, Doungla, Smout, Van Loey & Hendrickx, 2006).
Ca\(^{2+}\)-pectin gel networks are generated via “junction zones”, whose mechanism of formation is mainly based on the “egg-box” model of Grant and his co-workers (1973). This model was used to describe the interaction between some polysaccharides and Ca\(^{2+}\), namely alginate (Morris, Rees, Thom & Boyd, 1978) and subsequently pectin (Morris, Powell, Gidley & Rees, 1982; Powell, Morris, Gidley & Rees, 1982). Junction zone formation through the “egg-box” model entails a cooperative binding of Ca\(^{2+}\) to contiguous non-methylesterified galacturonic acid (NM-GalA) residues (referred to as “block”) of adjacent pectin molecules. The extent of cooperativeness depends on the number of contiguous NM-GalA residues involved in the formation of individual junction zones. So far, the minimum number of residues required for the formation of highly cooperative or stable junction zones has been rather controversial. Depending on the models used by various research groups, it has been estimated to be 14 (Powell et al., 1982), 9 (Liners, Thibault, & Vancutsem, 1992), 20 (Braccini & Perez, 2001) or 6 (Luzio & Cameron, 2008). “Large” NM-GalA blocks cooperatively form “strong associations” with Ca\(^{2+}\) (Powell et al., 1982) while short blocks are involved in less cooperative or “less specific interactions” with Ca\(^{2+}\) (Durand, Bertrand, Busnel, Emery, Axelos, Thibault, Lefebvre, Doublier, Clark, & Lips, 1990). The size and thus the distribution of NM-GalA blocks on pectin molecules might play a major role in defining the nature (strength / stability) and number of junction zones occurring in pectin-Ca\(^{2+}\) networks. Some mechanical characteristics of polymer gels can be related to the nature and number of junction zones. This suggests that the pattern of methylester distribution greatly influences the characteristics of pectin gels (Speiser, Copley & Nutting, 1947). A number of researchers provided practical evidence based on works mostly carried out
on LMP (Powell et al., 1982; Ström, Ribelles, Lundin, Norton, Morris, & Williams, 2007; Cárdenas, Goycoolea & Rinaudo, 2008; Fraeye, Doungla, Duvetter, Moldenaers, Van Loey & Hendrickx, 2009). Depending on the methylester distribution pattern, some HMPs might also be predisposed to form Ca$^{2+}$ gels. Studies on the rheological characteristics of Ca$^{2+}$-HMP gels have been limited to the influence of PMEs (plant and fungal) de-esterified polymers on networks characteristics (Kim & Wicker, 2009; Vincent & Williams, 2009). The effects of the extent and mechanism of de-esterification on most rheological characteristics of pectin gels remains largely unexplored.

Substantial insight in the rheological characteristics of Ca$^{2+}$-pectin gels requires an in depth assessment of both Ca$^{2+}$-LMP and Ca$^{2+}$-HMP gels. Besides, while using pectin as texture “improver” (due to its Ca$^{2+}$ gelling ability) be it in planta or as additive, the stability and nature of induced Ca$^{2+}$-pectin networks might be of great importance to food scientists, technologists and industries. Extensive studies on these rheological characteristics of pectin gels might enable to clearly identify those critical features of pectin responsible for a specific network character. Therefore, the present research aimed at investigating the effect of the degree and pattern of methylesterification on network development and nature of pectin-Ca$^{2+}$ gels. Consequently, starting from parent pectin of very high DM, partially de-esterified pectins of various degrees and patterns of methylesterification (PM) were produced and characterized using state-of-the-art methods. Afterwards, pectin gels were prepared at varied Ca$^{2+}$ concentration. The responses of these gels to dynamic rheological tests were related to the structural characteristics of the polymer.
2. Materials and methods

2.1 Materials

A commercial liquid preparation of recombinant *Aspergillus aculeatus* PME purchased from Novozymes was purified by gel filtration chromatography (Duvetter, Van Loey, Smout, Verlent, Nguyen, & Hendrickx, 2005).

Carrot (*Daucus carota var. Nantes* of Belgian origin) PME was extracted and purified using affinity chromatography (Jolie, Duvetter, Houben, Clynen, Sila, Van Loey, & Hendrickx, 2009).

Pure endo-PG from *Kluyveromyces fragilis* was kindly provided by the Laboratory of Food Chemistry of the Wageningen University.

Esterified citrus pectin (DM~94%, GalA content ~85% on dry basis) was purchased from Sigma-Aldrich (P956, lot 077K1432) and encoded M94. Pectins with varying degree and pattern of methylesterification were produced by controlled partial de-esterification of M94 using carrot PME (P-pectins), *A. aculeatus* PME (F-pectins) or NaOH saponification (C-pectins). Detailed production procedures of various PDPs have been described elsewhere (Fraeye et al., 2009; Ngouémazong, Tengweh, Duvetter, Fraeye, Van Loey, Moldenaers & Hendrickx, 2010). Subsequently, all pectins were extensively characterized as described in Ngouémazong et al. (2010).

All chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of Ca^{2+}-pectin gels

For gel preparation, each PDP sample was dissolved in Milli-Q water (18ΩM) at 4
°C and the pH of the solution was adjusted from ~4.5 to 6.0 (± 0.05) using NaOH solutions of concentrations varying from 1.0 to 0.1 M, and under continuous vigorous stirring. The resulting solution was immediately frozen and stored at -40 °C till gel preparation. As the different PDP samples had slightly different GalA content (mol/g pectin; dry matter basis) as a result of de-esterification, the concentration of the pectin solutions was adjusted to constant GalA content (1.68% w/v i.e. 1.68% GalA of unit weight as GalA, 194.14). This correction resulted in an average pectin concentration of 2% w/v (between 1.96 and 2.04% w/v).

A 3M CaCl₂ solution prepared using Milli-Q water was used as CaCl₂ stock solution. Prior to gel preparation, this stock solution was diluted so as to prepare gels with defined Ca²⁺ concentration expressed as the stoichiometric ratio (R= \(2[\text{Ca}^{2+}] / [\text{COO}^-]\)), while adding a constant volume of CaCl₂ solution. Therefore, the concentration of the CaCl₂ solution varied with the DM of PDP samples and the gel R-value. Since the calcium content of a 40% (w/v) M94 solution was checked and found to be negligible (< 0.00005%), the R-value of each prepared gel was varied between 0.0 (no CaCl₂ added) and ~5.7. The GalA content of all gels was ~1.53% w/w.

A technique which involved the combination of mild heating and diffusion of Ca²⁺ through microlitres of pectin was used for Ca²⁺-pectin gel preparation. In this method, Ca²⁺-pectin gels were prepared on the lower plate of a stress-controlled Physica MCR 501 rheometer (Anton Paar, Austria) (Doungla, Vandebril, Duvetter, Van Loey, Moldenaers & Hendrickx, 2009). More specifically, few microliters of pectin and CaCl₂ solutions were pre-heated to 50 °C and 30 °C respectively. The Peltier controlled lower plate of the rheometer was preheated to 50 °C. Exactly 262 µl of preheated pectin
solution was placed at the centre of the lower plate. Subsequently, 28 µl of preheated CaCl$_2$ was added drop-wise (14 droplets of 2 µl each) over the entire pectin surface. The upper geometry (25 mm Ø parallel plate) was then lowered to the set measuring gap (0.5 mm). The sample surface was covered with light paraffin oil to prevent evaporation during gel development and measurements. In order to limit temperature fluctuations within the gel sample, a Peltier controlled hood was covering the loaded sample.

### 2.2.3 Small-amplitude oscillatory shear tests

Once loaded (time zero of the experiment), the Ca$^{2+}$-pectin mixture was allowed to equilibrate for 10 min (to ensure among others complete calcium diffusion through the 262 µl pectin sample) after which it was cooled to 20 °C (0.5 °C/min) for 1 h. The gel was subsequently let to evolve for 5 h at 20 °C. For some gels, prolonged ageing has been studied. Preliminary stress sweep tests (at 1 rad/s) were carried out on a number of Ca$^{2+}$-pectin systems in order to define the linear viscoelastic (LVE) region of the gels. In this region, the viscoelastic response of the sample (storage modulus (G’) and loss modulus (G’’)) is independent of the applied stress amplitude. All oscillatory shear tests were carried out within the LVE region, thereby excluding effects of shear-induced gelation or structure break-down. The network development of various Ca$^{2+}$-pectin systems was assessed by means of a time sweep test (angular frequency: 1 rad/s), carried out during cooling followed by isothermal conditions. Thereafter, a frequency sweep test (0.1-10 rad/s) was performed so as to investigate the frequency dependence of the moduli of the gels. Although the gel preparation method and rheological test
results have been reported to be very reproducible (Doungla et al., 2009), most gel samples were analysed in duplicate.

3. Results and discussion

3.1 Summary of structural characteristics of partially de-esterified pectins

M94 was partially de-esterified to a wide range of DM using carrot PME (P-pectins), *A. aculeatus* PME (F-pectins) or NaOH saponification (C-pectins). Although an extensive characterization of PDPs has been reported earlier (Ngouémazong et al., 2010), an understanding of Ca$^{2+}$-pectin gels behaviour requires an overview of several pectin structural features. None of the PDP samples show a shift in elution time as compared to the mother pectin on HPSEC chromatograms, confirming that none of the produced pectins was depolymerised during the de-esterification process.

Extensive hydrolysis of PDPs using endo-PG from *K. fragilis* yielded non-methylesterified galacturonides (as mono- di- and tri-galacturonic acid (NM-MDT-GalA)) and methylesterified galacturonides. The determination of the NM-MDT-GalA released enabled to calculate the total non-methylesterified GalA released, which forms the basis for $\text{DB}_{\text{abs}}$ estimation. $\text{DB}_{\text{abs}}$ is estimated as the ratio of the total non-methylesterified GalA released to the total galacturonic acid of the polymer (Ngouémazong et al., 2010). Hence, $\text{DB}_{\text{abs}}$ gives an indication of the absolute occurrence of NM-GalA residues in blocks over the entire PDP, thus portraying the methylester distribution on the polymer.

Table 1 displays the DM and $\text{DB}_{\text{abs}}$ of the produced PDPs. Generally, $\text{DB}_{\text{abs}}$ increased with decreasing DM, irrespective of the de-esterification method. This is an indication that de-esterification causes an increase in the total amount of non-
methylesterified GalA released. As it is hypothesized that at least four consecutive NM-GalA residues are required to ensure *K. fragilis* endo-PG hydrolysis of PDPs (Pasculli, Geraeds, Voragen & Pilnik, 1991), an increase in the amount of non-methylesterified galacturonides released is suggested to result from an increase in the size and/or number of NM-GalA blocks occurring on PDPs. Therefore, de-esterification results in an increase in the size and/or number of NM-GalA blocks. At similar DM, DB$_{abs}$ values varied with de-esterification mechanism, with saponification showing the lowest DB$_{abs}$ value. This implies that the size / number of NM-GalA blocks occurring on PDPs depend on the de-esterification mechanism, which is consistent with the described mechanisms of methylesters hydrolysis. Specifically, these esters are either randomly hydrolysed as in saponified C-pectins or sequentially hydrolysed or as in block-wise de-esterified F- and P-pectins (Hotchkiss, Savary & Cameron, 2002), with P-pectins showing the highest extent of sequential hydrolysis.

As discussed by Ngouémazong et al. (2010), assessment of the proportions of NM-MDT-GalA released by endo-PG revealed that at DB$_{abs}$ ≥ 33%, 31% or 19% (corresponding to DM ≤ 57%, 37% or 35%) in P-, F- and C-pectins respectively, tri-GalA shows the highest proportion (~60%). Similar proportions of tri-GalA were also reported in the endo-PG digest of the methylester free polymer, poly-D-GalA (Ngouémazong et al., 2010). These results suggest the occurrence of large NM-GalA blocks on the aforementioned PDPs. This is in accordance with Daas and his co-workers (2000) who relate a high proportion of tri-GalA to the presence of large NM-GalA blocks on the polymer.
3.2 Effect of de-esterification and Ca\textsuperscript{2+} concentration on the structure development and nature of Ca\textsuperscript{2+}-pectin gels

PDP solutions (pH 6.0) were used for the preparation of Ca\textsuperscript{2+}-pectin gels of varying R-values. During gel formation, time sweep tests were carried out to assess the network development in various gels. With small amplitude oscillatory shear (as in time sweep tests), the time-dependent change of the gel strength can be monitored by means of the evolution of the storage modulus (G') with time (Mezger, 2006). In the present study, some networks developed rather instantly, hence kinetic studies of the gelation process and structure development could not be carried out. A qualitative mechanistic discussion of the major interactions involved in network formation was considered, based on the structural features of pectin, the network development profile and final strength of the gels.

Although defining the nature of gels has been a source of contradiction and misunderstanding (Nishinari, 2009) an attempt was made, based on some fundamental criteria to classify PDPs-Ca\textsuperscript{2+} gels (after 5 h at 20° C, unless otherwise). Evaluation of the gels mechanical spectra enabled an assessment of the frequency-dependent character ("weak" or "true" gels) of pectin gels (Rao, 1999; Mezger, 2006). Monitoring the tanδ (ratio of G" to G') throughout the set frequency range (0.1-1.0 rad/s) allowed the evaluation of the elastic character of the gels (Lopes da Silva & Rao, 1999; Mezger; 2006). In addition, as suggested by Nishinari (2009), gel strength was used as a basis to refine PDP gels classification.
3.2.1 Development and nature of networks in concentrated pectin solutions

Prior to the assessment of Ca\(^{2+}\)-pectin gels, concentrated pectin solutions (~2% (w/v)), (no Ca\(^{2+}\) added i.e. R=0.0) were evaluated. The mother pectin (M94) and all PDP solutions were subjected to similar thermal history as Ca\(^{2+}\)-pectin systems. Fig. 1 depicts the structure development of concentrated pectin solutions. Generally, during non-isothermal followed by isothermal conditions, the storage modulus (G') was greater than the loss modulus (G''), indicating the formation of a network. All PDP solutions displayed similar profiles as those of M94 (Fig. 1a) and C10 (Fig. 1b). In addition, the mechanical spectra of concentrated pectin solutions revealed both storage modulus (G') and loss modulus (G'') almost parallel to each other and highly frequency dependent throughout the set frequency range (0.1-10 rad/s) (Fig. 2, for M94 with R=0.0).

Generally, in concentrated polymer solutions, chains come in contact with each other. Hence, their motion becomes restricted due to entanglements. Decrease of temperature further hinders polymer chain mobility and promotes H-bonding (Lopes da Silva, Gonçalves, & Rao, 1995) thus causing the sharp increase in G'. Owing to the occurrence of some hydrophobic (methyl) groups in pectins, it might be suggested that hydrophobic interactions also contribute to network development. However, throughout isothermal rest, the strength of M94 (high methyl group content) was rather similar to that of C10 (low amount of hydrophobic moieties). This suggests a weak contribution of hydrophobic interactions to the network strength. Since the Ca\(^{2+}\) content of the starting pectin was checked and found to be below detection limit (results not shown), network formation in concentrated pectin solutions could not be attributed to junction zone formation.
The high frequency dependence of the dynamic moduli suggests that, in pectin solutions, network relaxation processes occur at relatively short time scales (Rao, 1999). In addition, the low elastic character ($\tan \delta \sim 0.3$) displayed by all networks is an indication that a high percentage of stored energy was lost in viscous dissipation due to molecular mobility (Mezger, 2006; Lopes da Silva & Rao, 1999). The fast relaxation of the network coupled with its inability to recover stored energy due to polymer chain mobility are striking features that have been associated with “weak” gels (Ross-Murphy, Morris, V. & Morris, E., 1983; Lopes da Silva & Rao, 1999; Mezger, 2006). Therefore, concentrated pectin solutions revealed “weak” gel-like characteristics. This is consistent with Clegg (1995) who reported that some concentrated polymer solutions respond to frequency sweep tests as gels. In these highly entangled networks, the transition frequency (frequency at which a transition from predominantly liquid-like response to a predominantly solid-like response is observed) often does not appear in the experimental window (Clegg, 1995; Lefebvre & Doublier 2005; Nishinari, 2006). Consequently, concentrated polymer solutions were considered as highly entangled networks in which entanglements can be partially immobilised due to H-bridges.

3.2.2 Structure development and nature of Ca$^{2+}$-pectin gels produced from partially de-esterified pectins of very high DM

PDPs of very high DM (C75, F80 and P84) were used for the production of gels with varying Ca$^{2+}$ concentrations (R-value). All C-pectin networks (low and high Ca$^{2+}$ concentrations) as well as F- and P- pectin gels with low Ca$^{2+}$ concentrations ($R \leq 0.3$) showed similar characteristics as those of concentrated pectin solutions (Fig. 2).
However, at higher Ca\(^{2+}\) concentration (R ≥ 2.0), some differences in gel characteristics were observed for gels produced from F- and P-pectins. The elastic character of the gels was improved (tanδ decreased from ~0.3 to ~0.15, from low to high Ca\(^{2+}\) concentration) (Fig. 2b and c). This was coupled with a somewhat reduced frequency dependence of the moduli, as well as a slight increase of the gel strength.

C-pectins of very high DM are characterised by very low DB\(_{abs}\) values (DB\(_{abs}\) ≤ 1%). This suggests the occurrence of sparsely distributed NM-GalA residues or/and extremely short blocks on numerous pectin chains (Ngouémazong et al., 2010) which could not stimulate gel formation, even at high Ca\(^{2+}\) concentration. On the other hand, F- and P-pectins of very high DM are characterised by the presence of some sparingly distributed short NM-GalA blocks (DB\(_{abs}\) ~4%) on the polymers (Ngouémazong et al., 2010). This suggests that at low Ca\(^{2+}\) concentration, limited cation availability coupled with the occurrence of short NM-GalA blocks on pectins might have caused the network to be formed by polymer chain entanglements rather than cross-linking. At higher Ca\(^{2+}\) concentration, the “improved” network characteristics indicate a conversion from an entangled network into a cross-linked network. At higher Ca\(^{2+}\) concentration, the presence of short NM-GalA blocks on F- and P-pectins of very high DM stimulates the formation of junction zones, albeit unstable and less cooperative. Nevertheless, they provide a means for cross-linking the entangled network (MacDougall, Needs, Rigby, & Ring, 1996). Still, the strength of the produced gels was very low (G’ ≤ 10 Pa). It has been proposed that “weak” gels which have very low storage moduli (G’ ≤ 10 Pa) should be referred to as a “structured liquid” (unpublished lecture notes of Ross-Murphy, 2008; Nishinari, 2009). Since F- and P-pectins of very high DM show similar average DM and
DBabs values (Table 1), the similarities observed in the characteristics of gels prepared from these pectins might suggest that they also reveal similar inter-molecular distribution of methylesters, unless the latter pectin feature does not influence gel characteristics.

It is worth noting that, as pectin is fully ionized at pH 6.0, some electrostatic interactions between Ca$^{2+}$ ions and the sparsely distributed NM-GalA residues or/and short blocks (depending on PM) might have also played a role in the network development. Generally, at high R-value, these interactions play a role in dimer aggregation whenever junction zones are involved in network development (Morris et al., 1982; Powell et al., 1982; Braccini & Pérez, 2001).

In conclusion, Ca$^{2+}$-pectin mixtures prepared from C-pectins of very high DM (DM~75%) displayed the characteristics of highly entangled networks, while Ca$^{2+}$ mixtures of F- and P-pectins of very high DM (DM~80 and ~84% respectively) showed this behaviour only at low Ca$^{2+}$ concentration. At high Ca$^{2+}$ concentration, Ca$^{2+}$-pectin gels prepared from the latter PDPs displayed the characteristics of “structured liquids”, whose structural build up was likely ascribed to less cooperative junction zone and electrostatic interactions.

3.2.3 Structure development and nature of Ca$^{2+}$-pectin gels produced from C- and P-pectins of high DM

PDPs of rather high DM (C66 as well as P77 and P63) were used for the preparation of gels with varying Ca$^{2+}$ concentrations. At low Ca$^{2+}$ concentration (R=0.25 and R=0.5), Ca$^{2+}$-C66 networks showed similar behaviour (in all characteristics,
including G') as those formed by Ca$^{2+}$-C-pectins of very high DM (see 3.2.2). At high Ca$^{2+}$ concentration (R > 0.5), a general increase of the gel strength was observed (Fig. 3a). Although these gels revealed a better elastic character (tanδ~0.15) as compared to low Ca$^{2+}$ gels, their moduli still displayed some dependence on frequency (results not shown). Contrary to C-pectin gels, all Ca$^{2+}$ gels produced from P-pectins of rather high DM were characterized by a somewhat high gel strength (2400 > G' > 100 Pa) and very good elastic character (tanδ~0.07). Under isothermal conditions, the strength of various gels increased slowly toward pseudo-equilibrium (Fig. 3b). Similar profiles were obtained for gels produced from P77. Nonetheless, as for high Ca$^{2+}$-C66 gels, the dynamic moduli of P-pectin gels displayed some dependence on frequency (results not shown), indicating the "weak" nature of these gels.

C66 is characterized by a low DB$_{abs}$ (5.5%), low proportions of NM-tri-GalA liberated and high proportions of NM-mono-GalA liberated, hence the presence of short NM-GalA blocks on the polymer (Ngouémazong et al., 2010). This suggests that the inability of C66 to form gels at low Ca$^{2+}$ concentration is mostly related to the limited availability of the cations. At high Ca$^{2+}$ concentration, the increased availability of the cations enhanced the cross-linking of NM-GalA blocks (Mac Dougall et al., 1996), resulting in the formation of numerous short junction zones, thus the generation of gels of relatively high strength (G'~160 Pa). Since short junction zones are less specific and weak ionic interactions (Durand et al., 1990; Cárdenas et al., 2008), they were likely responsible for the limited expression of other gel characteristics.

The PDPs used for the preparation of the P-pectin gels revealed relatively high DB$_{abs}$ values (Table 1) and high proportions of di- and tri-GalA (Ngouémazong et al.,
Such proportions of di- and tri-GalA have been associated with the occurrence of NM-GalA blocks of intermediate size on the polymer (Daas, Voragen, & Schols, 2000). From the onset of gelation, the high gel strength observed in P-pectin gels might indicate rapid formation of junction zones. The occurrence of large NM-GalA blocks on pectin stimulates rapid and strong “associations” through cooperative binding of Ca\[^{2+}\] to adjacent NM-GalA blocks (Morris et al., 1982; Powell et al., 1982). This implies that the size of the NM-GalA block on P-pectins (DM~77 and 63%) was large enough to stimulate the generation of strong cooperative junction zones. The high capability of the network to retain stored energy (high gel elastic character) corroborates the high strength of the network interactions.

Therefore, at low R-values, Ca\[^{2+}\]-C66 systems are entangled networks while at high R-values, less cooperative junction zones played a major role in the formation of “weak” gels. In the presence of Ca\[^{2+}\], P-pectins of relatively high DM (DM~77 and 63%) produced “weak” gels whose network development is mostly ascribed to cooperative junction zone formation.

### 3.2.4 Structure development and nature of Ca\[^{2+}\]-pectin gels produced from C-pectins of low DM and F-pectins of high and low DM

C-pectins of low DM (C48, C35 and C26) as well as F-pectins of high and low DM (F64, F45, F37, F29) were used for the preparation of gels with varying Ca\[^{2+}\] concentrations. Generally, regardless of the degree and pattern of methyl esters distribution, the network development of Ca\[^{2+}\] gels produced from these PDPs revealed
a similar profile (Fig. 4a, example of Ca\textsuperscript{2+}-C48 gels). Irrespective of the R-value, from the onset of gel formation, Ca\textsuperscript{2+} gels of these PDPs revealed high gel strength. Although all tests were carried out within the linear viscoelastic region, the strength of low Ca\textsuperscript{2+} gels usually decreased during isothermal conditions. A similar profile has been reported in literature for a Ca\textsuperscript{2+} gel prepared (at pH 7.0 and 20°C) from a randomly de-esterified pectin (DM~61%) and at low Ca\textsuperscript{2+} concentration (Kim & Wicker, 2009). However, at high Ca\textsuperscript{2+} concentrations (usually R > 0.5), the networks developed to quasi-equilibrium, within the observation time of 5 h. Although all high Ca\textsuperscript{2+} gels displayed rather frequency independent moduli (results not shown), F-pectin gels revealed higher elastic character as compared to C-pectin gels (tanδ~0.08 and tanδ~0.1 for F- and C-pectin gels respectively).

The PDPs whose gels showed a decrease of the gel strength with time are usually characterised by DB\textsubscript{abs} values varying from 9% to 37% and 9% to 31% for C- and F-pectins respectively. It is rather striking that these pectins are randomly de-esterified polymers which reveal similar DB\textsubscript{abs} values (except F29 (DB\textsubscript{abs}~50%) whose low Ca\textsuperscript{2+} (R = 0.25) gel showed a hardly noticeable decrease of G\textsuperscript{'} with time). In randomly de-esterified pectins, methylesterified carboxyl groups are randomly distributed over the molecule. This implies that within a molecule, stretches of NM-GalA residues occur intermittently with stretches of methylesterified GalA residues. Stretches of methyl groups have been reported to form steric hindrance during Ca\textsuperscript{2+}-pectin gelation (Powell et al., 1982; Axelos, Lefebvre, Qui & Rao, 1991). These hindrances obstruct cooperative binding of Ca\textsuperscript{2+} over the entire NM-GalA blocks, resulting in random binding of the cations over easily accessible binding sites. At low Ca\textsuperscript{2+}
concentration (depending on DM and PM of PDPs), the limited availability of Ca\(^{2+}\) results in the partial filling of the NM-GalA blocks. Consequently, the networks bearing partially filled NM-GalA blocks are characterized by the prevalence of short junction zones. So far, short junction zones which consist of less specific and weak ionic interactions have been reported to be unstable (Durand et al., 1990), hence susceptible to rearrangements. Low gel strength is generally associated with low number of network cross-link points (or junction zones in the case of Ca\(^{2+}\)-pectin gels). This implies that during ageing of Ca\(^{2+}\)-pectin gels, some processes continuously reduced the number of network junction zones thereby continuously decreasing gel strength. As the isothermal conditions (20 °C) did not pre-dispose Ca\(^{2+}\)-pectin networks to either Ca\(^{2+}\) chelation or biopolymer hydrolysis, it might be suggested that the decrease in the number of junction zones is inherent to the network rearrangements, as a result of junction zones instability.

In cooperative junction zone formation, the binding of the first Ca\(^{2+}\) cation facilitates the binding of the next ion, and so on along the NM-GalA blocks (Cabrera, Boland, Messiaen, Cambrier, & Van Cutsem, 2008). The formation of stable cooperative junction zones is then less energy requiring. During ageing of Ca\(^{2+}\) gels produced from the abovementioned PDPs, it is proposed that Ca\(^{2+}\) present in less cooperatively bound, weak and unstable short junction zones is re-organized between large or intermediate size NM-GalA blocks via cooperative binding to yield energetically stable or larger junction zones, whose number is obviously lower than the number of the unstable or shorter ones (Fig. 5). The lower number of newly formed junction zones then results in a decrease in gel strength. The stability of these newly formed junction zones depends,
aside from Ca$^{2+}$ concentration, on the size of the largest NM-GalA blocks occurring on PDPs. In case of PDPs with relatively short NM-GalA blocks which can not generate stable junction zones in the presence of Ca$^{2+}$ (as C48; DB$_{abs}$$\sim$9%), continuous re-arrangements of the new less unstable junction zones will proceed. Yet, such re-arrangements might not have any further effect on the network structural strength. This proposed Ca$^{2+}$ binding mechanism further implies that, the time required to achieve the formation of large or stable junction zones depends on the number and / or size of partially filled NM-GalA blocks (whose Ca$^{2+}$ are susceptible to be re-organized) as well as Ca$^{2+}$ concentration. Furthermore, the structural strength of the network which bears stable junction zones equally depends on the number and / or size of the unfilled NM-GalA blocks, as well as Ca$^{2+}$ concentration. Generally, prolonged ageing results in less (albeit more stable) junction zones, hence a more stable network with lower strength. The proposed model and its implications are in agreement with the structure development profiles of low Ca$^{2+}$ gels produced from C35 and F29 (Fig. 4b). Under prolonged isothermal conditions, the structural strength of these gels decreased up to a rather constant value which might be associated with the formation of a network consisting of stable junction zones. Although theses PDPs revealed high proportions of NM-tri-GalA, their molecules still carried some stretches of methylesters which hindered cooperative binding of Ca$^{2+}$. At R=0.25, the gel produced from F29 (DB$_{abs}$~50%) showed some indications of stabilization after about 20 hours of rest whereas, the gel strength of Ca$^{2+}$-C35 (DB$_{abs}$$\sim$19%) gel still displayed some slight continuous decrease. This indicates the formation of relatively large junction zones in F29 gels, which required the addition of fewer Ca$^{2+}$ in order to be stabilized, suggesting the occurrence of NM-
GalA blocks of larger size and thus shorter blocks of methylesters on F29 molecules, as already confirmed by DB_{abs} value. Nonetheless, as the exact size of junction zones or NM-GalA blocks has not yet been determined, need arises to carry out further studies on the network development of Ca^{2+} gels prepared from PDPs of C- and F-pectins with intermediate DM.

The relatively low damping factor (tanδ~0.1) observed in high Ca^{2+}-C50-C35 gels was indicative of the “improved” elastic character of these gels. This was likely due to the formation of longer / stable junction zones associated with the filling (through cooperative binding) of NM-Gal blocks with available Ca^{2+} ions. Consequently, processes which ensure the generation of stable or less unstable Ca^{2+} junction zones will yield gels with improved elastic character. This is in agreement with prolonged ageing (characterised by rearrangement of less cooperatively bound Ca^{2+} to yield stronger stable or less unstable junction zones), which fairly improves gel elastic character, as illustrated (Fig. 6a) for Ca^{2+}-F29 networks whose damping factor was slightly decreased (from 0.07 to 0.04) after 60 h of ageing. Nevertheless, it is worth noting that, if the process of improving junction zone strength (thus stability) causes a drastic reduction of the number of polymer chains involved in cross-links (as during the ageing of low Ca^{2+}-C35 gels), the resulting gels will show very high polymer chain mobility hence, further increased frequency dependence of the moduli (Fig. 6b).

In conclusion, at low Ca^{2+} concentration, the continuous decrease in the gel strength for gels (either “weak” or “true” depending on DM and PM) prepared from C-pectins (DM~48, 35 and 26%) and F-pectins (DM~64, 45, 37 and 29%) might be due to the formation of short unstable junction zones, that are re-organised with prolonged
ageing into less unstable or stable junction zones. At high Ca\(^{2+}\) concentration on the other hand, network development in “true” gels is mostly ascribed to the formation of cooperative or less cooperative (depending on PM) junction zones and electrostatic interactions. Nevertheless, pectin side chains might influence the mechanism proposed in this study, as the work was carried out on a polymer with limited branching.

3.2.5 Structure development and nature of Ca\(^{2+}\)-pectin gels produced from P-pectins of intermediate DM as well as C-, F- and P-pectins of very low DM

Gels of varying Ca\(^{2+}\) concentration were prepared using P-pectins (P57, P47, P37, P18 and P11) as well as C- and F-pectins of very low DM (F15 and C10 respectively). Regardless of R-values, all gels showed high gel strength (40 ≤ G’ ≤ 60,000 Pa) from the onset of gel formation (results not shown). The dynamic moduli of Ca\(^{2+}\)-pectin gels produced from PDPs of low DM (including P57) displayed a negligible dependence on frequency, at all Ca\(^{2+}\) concentration studied, thereby revealing a “true” gel nature. In addition, all gels displayed high elastic character (average tanδ~0.07 for F- and C- pectin gels; average tanδ~0.05 for P-pectin gels).

The PDPs used for gel preparation are characterised by low DM values (corresponding to 40% < DB\(_{abs}\) < 85% and high proportions of NM-GalA liberated) which suggests the occurrence of long blocks of NM-GalA on the polymers (Ngouémazong et al., 2010). The occurrence of long NM-GalA blocks on PDPs stimulate rapid cooperative binding of Ca\(^{2+}\) so as to produce energetically stable and strong junction zones, hence gels with pronounced elastic character. Besides, the low DM and/or the PM of the aforementioned PDPs suggest the occurrence of long NM-GalA blocks on many pectin
chains (Ngouémazong et al., 2010). The involvement of many pectin chains in the formation of many strong junction zones results in denser and stronger networks. In these networks, the high number of dimerized polymer chains reduces polymer chain mobility, hence results in the formation of “true” gels. Consequently, both the strength of the junction zones (size of NM-GalA blocks) and the number of Ca\(^{2+}\) cross-linked (dimerized) chains involved in network formation (inter-molecular distribution of NM-GalA blocks thus distribution of junction zones within the network) play an important role in ensuring gel characteristics, via gel elastic character and network chain mobility respectively. Apart from that, cooperatively bound Ca\(^{2+}\) yields stable junction zones (Fig. 7) which generates stable networks when many chains are involved in network formation.

Therefore, regardless of Ca\(^{2+}\) concentration as well as intra- and inter-chain pattern of methylester distribution of low methylesterified PDPs (including P57), all networks were generated through cooperative junction zone formation and yielded “true” gels.

### 3.3 Conclusion

Ca\(^{2+}\)-pectin gels were characterized based on network development with time, gel strength and gel elastic character. Generally, gel characteristics varied with Ca\(^{2+}\) concentration (R-value) and with pectin structural features, namely DM and DB\(_{abs}\), as summarised in Table 2. In the presence of sufficient Ca\(^{2+}\), pectins with high DB\(_{abs}\) values (thus, large NM-GalA blocks) produced strong and stable cooperative junction zones (hence, networks which develop toward pseudo-equilibrium), while polymers with
low DB\textsubscript{abs} values (thus, short NM-GalA blocks) generated in most cases less cooperative and weak junction zones, and consequently, evolving networks. Besides, the occurrence of large NM-GalA blocks on the polymer stimulated the generation of strong network interactions, thereby resulting in the formation of gels with very good elastic character. Furthermore, the distribution of NM-GalA blocks on many polymer chains enabled the Ca\textsuperscript{2+} dimerisation of several polymer chains. The latter resulted in the generation of networks with reduced polymer chain mobility and thus, the formation of “true” gels.

Evidently, pectin structural features and Ca\textsuperscript{2+} concentration govern the characteristics of Ca\textsuperscript{2+}-pectin gel networks. Consequently, at specific R-values, qualitative structure-function relations of pectins can be established. These relations enable to gain insight into Ca\textsuperscript{2+}-pectin gels and may constitute a stepping stone for the development of quantitative structure-function relations. In addition, applications of the scientific knowledge acquired from this study may not only allow improving fruit / vegetable processing, but also enhance or stimulate the well-considered use of LMP or naturally occurring HMP as functional ingredients for the production of low-calorie healthy foods.
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Table 1: DM and DB$_{abs}$ of PDPs. Results without standard deviation are from single analysis.

<table>
<thead>
<tr>
<th>Pectin</th>
<th>Sample code</th>
<th>Amount of methylesters (DM (%))</th>
<th>Quantification of methylesters distribution (DB$_{abs}$ (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent citrus</td>
<td>M94</td>
<td>94.2±0.2 a</td>
<td>&lt; d.l.</td>
</tr>
<tr>
<td>P-pectins</td>
<td>P84</td>
<td>84.1±0.2</td>
<td>4.5±2.3</td>
</tr>
<tr>
<td></td>
<td>P77</td>
<td>76.9±0.1</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>P63</td>
<td>63.4±0.2</td>
<td>25.7</td>
</tr>
<tr>
<td></td>
<td>P57</td>
<td>57.02</td>
<td>33.1</td>
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<td></td>
<td>P47</td>
<td>47.4±0.2</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td>P37</td>
<td>37.4±0.3</td>
<td>50.9</td>
</tr>
<tr>
<td></td>
<td>P18</td>
<td>17.6±0.3</td>
<td>79.8±3.2</td>
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<td>P11</td>
<td>10.9±0.1</td>
<td>82.5±0.8</td>
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<tr>
<td>F-pectins</td>
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<td>80.2±0.2</td>
<td>3.7</td>
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<td>F64</td>
<td>64.2±0.1</td>
<td>8.6</td>
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<td>F45</td>
<td>44.9±0.4</td>
<td>23.3±1.2</td>
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<td></td>
<td>F37</td>
<td>36.4±0.5</td>
<td>31.3±2.9</td>
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<td>F29</td>
<td>28.6±0.2</td>
<td>50.0±0.9</td>
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<td>F15</td>
<td>14.9±0.3</td>
<td>79.4±1.9</td>
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<tr>
<td>C-pectins</td>
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<tr>
<td></td>
<td>C75</td>
<td>75.5±0.2</td>
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<td></td>
<td>C66</td>
<td>66.4±0.4</td>
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</tr>
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<td>C48</td>
<td>48.4±0.1</td>
<td></td>
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<tr>
<td></td>
<td>C35</td>
<td>35.4±0.2</td>
<td></td>
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<tr>
<td></td>
<td>C26</td>
<td>26.3±0.2</td>
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<tr>
<td></td>
<td>C10</td>
<td>10.1±0.3</td>
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</tr>
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<td></td>
<td></td>
<td>0.26±2.5</td>
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<td></td>
<td></td>
<td>5.5±2.1</td>
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<td>8.5±0.9</td>
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<td>19.2±1.1</td>
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<td>37.0±0.8</td>
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<td></td>
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<td>64.4±1.3</td>
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</tr>
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</table>

*a*: standard deviation; d.l.: detection limit
Table 2: Summary of Ca\textsuperscript{2+}-pectin gels characteristics

<table>
<thead>
<tr>
<th>Pectins</th>
<th>DM (%)</th>
<th>DB\textsubscript{abs} (%)</th>
<th>Gel strength with time</th>
<th>Most important network “interactions”</th>
<th>Nature of networks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>94.2</td>
<td>&lt; d.l.\textsuperscript{a}</td>
<td>Increase</td>
<td>Polymer chain entanglements</td>
<td>Entangled networks; (tanδ~0.36; G'&lt;10 Pa)</td>
</tr>
<tr>
<td>Very high DM (3.2.2)</td>
<td>75.5</td>
<td>0.26</td>
<td>Increase</td>
<td>Polymer chain entanglements</td>
<td>Entangled networks; (tanδ~0.36; G'&lt;10 Pa)</td>
</tr>
<tr>
<td>High DM (3.2.3)</td>
<td>66.4</td>
<td>5.5</td>
<td>Increase</td>
<td>Polymer chain entanglements (\text{low R}) Less c.j.z.\textsuperscript{b} and electrostatic interactions (\text{high R})</td>
<td>Entangled networks; (tanδ<del>0.36; G'&lt;10 Pa) (\text{low R}) Weak gels; (tanδ</del>0.15; G'~163 Pa) (\text{high R})</td>
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<tr>
<td>Low DM (3.2.4)</td>
<td>48.4</td>
<td>8.5</td>
<td>Decrease (\text{low R}) less c.j.z. (\text{low R}) c.j.z. and electrostatic</td>
<td>True (strong) gels ; (tanδ~0.15 - ~0.1)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} C-pectin gels

\textsuperscript{b} c.j.z = covalent intermolecular interactions

\textsuperscript{c} DM = Degree of Methylation
<table>
<thead>
<tr>
<th></th>
<th>DM (%)</th>
<th>DB$_{abs}$ (%)</th>
<th>Gel strength with time</th>
<th>Most important network “interactions”</th>
<th>Nature of networks</th>
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</thead>
<tbody>
<tr>
<td><strong>Starting material</strong></td>
<td>94.2</td>
<td>&lt; d.l.$^a$</td>
<td>Increase</td>
<td>Polymer chain entanglements</td>
<td>Entangled networks; (tanδ~0.36; G’&lt;10 Pa)</td>
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<td><strong>Very high DM (3.2.2)</strong></td>
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<td>≤ 3.7</td>
<td>Increase</td>
<td>Polymer chains entanglements (low R)</td>
<td>Entangled networks; (tanδ~0.36; G’&lt;10 Pa) (low R)</td>
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<tr>
<td><strong>Very low DM (3.2.5)</strong></td>
<td>10.1</td>
<td>64.4</td>
<td></td>
<td>True (strong) gel; (tanδ~0.07; 19000&lt;G’&lt;50400 Pa) (low and high R)</td>
<td>Structured liquids (tanδ~0.14; G’&lt;10 Pa) (high R)</td>
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$^a$: detection limit  
$^b$: cooperative junction zone
<table>
<thead>
<tr>
<th>High and low DM (3.2.4)</th>
<th>64.2</th>
<th>8.6</th>
<th>Decrease (low R)</th>
<th>Less c.j.z. (low R)</th>
<th>True gels (tanδ~0.08; 90&lt;(G'&lt;25900 \text{ Pa})) (low and high R)</th>
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<td></td>
<td>44.9</td>
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<td>Increase to quasi-equilibrium (high R)</td>
<td>c.j.z. and electrostatic interactions (high R)</td>
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<tr>
<td></td>
<td>36.4</td>
<td>31.3</td>
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<td>28.6</td>
<td>50.0</td>
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<table>
<thead>
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<th>Very low DM (3.2.5)</th>
<th>14.9</th>
<th>79.4</th>
<th>Increase to quasi-equilibrium</th>
<th>c.j.z. (low R)</th>
<th>True (strong) gels (tanδ~0.07; 20000≤(G')≤64000 Pa) (low and high R)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>c.j.z. and electrostatic interactions (high R)</td>
<td></td>
</tr>
</tbody>
</table>

**a:** detection limit  
**b:** cooperative junction zone

**c:** P-pectin gels

<table>
<thead>
<tr>
<th>Pectins</th>
<th>DM (%)</th>
<th>(\text{DB}_{\text{abs}}) (%)</th>
<th>Gel strength with time</th>
<th>Most important network “interactions”</th>
<th>Nature of networks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting material</td>
<td>94.2</td>
<td>&lt; d.l.(^a)</td>
<td>Increase</td>
<td>Polymer chain entanglements</td>
<td>Entangled networks; (tanδ~0.36; (G')~10 Pa)</td>
</tr>
<tr>
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<td>Entangled networks (tanδ~0.36; (G')&lt;10 Pa) (low R)</td>
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<td>33.1</td>
<td>Increase to quasi-equilibrium</td>
<td>c.j.z. (low R)</td>
<td>c.j.z. &amp; electrostatic interactions (high R)</td>
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<tr>
<td>Low and very low DM (including P57) (3.2.5)</td>
<td>47.4</td>
<td>38.9</td>
<td>Increase to quasi-equilibrium</td>
<td>c.j.z. (low R)</td>
<td>c.j.z. &amp; electrostatic interactions (high R)</td>
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<tr>
<td>High DM (3.2.3)</td>
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<td>14.6</td>
<td>Increase to quasi-equilibrium</td>
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<tr>
<td></td>
<td>63.4</td>
<td>25.7</td>
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</tbody>
</table>

|                  |       |       | less c.j.z. (high R)         |               |                                             |                                                                 |

a: detection limit  
b: cooperative junction zone
Figure captions

Figure 1:
Structure development of concentrated pectin solutions (no \( \mathrm{Ca}^{2+} \) added). Black and grey symbols represent \( G' \) and \( G'' \) respectively. (a) and (b) represent the evolution of mother pectin and C10 (chemically de-esterified pectin with DM = 10%) solutions respectively. Tests carried out at frequency of 1 rad/s.

Figure 2:
Mechanical spectra of \( \mathrm{Ca}^{2+} \)-pectin gels prepared from PDPs of very high DM. Filled and open symbols represent \( G' \) and \( G'' \) respectively. (a), (b) and (c) enable to compare the mechanical spectrum of entangled networks to those of C-, P- and F-pectin gels respectively. Most often, standard errors are so small that they fall within the symbol size.

Figure 3:
Structure development of \( \mathrm{Ca}^{2+} \)-pectin gels produced from C- and P-pectins of high DM, at varying \( \mathrm{Ca}^{2+} \) concentrations. (a) and (b) represent gels prepared from C- and P-pectins respectively. Tests carried out at frequency of 1 rad/s.

Figure 4:
(a) Examples of structure development of \( \mathrm{Ca}^{2+} \)-pectin gels produced from C-pectins of low DM, at varying \( \mathrm{Ca}^{2+} \) concentrations. (b) Effect of prolonged ageing on the evolution
of Ca\textsuperscript{2+}-pectin networks. Black and grey lines represent G’ and G” respectively. Tests carried out at frequency of 1 rad/s.

Figure 5:
Suggested Ca\textsuperscript{2+} binding mechanism in PDPs whose gels showed a reduction of the gel strength with time.

Figure 6:
Effect of prolonged ageing on gel character of low Ca\textsuperscript{2+}-pectin gels (which showed a reduction of the gel strength with time). Filled and open symbols represent G’ and G” respectively.

Figure 7:
Suggested Ca\textsuperscript{2+} binding mechanism in PDPs bearing large NM-GalA blocks.
Figures

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Randomly de-esterified pectin: DM~27%

Random binding of Ca^{2+} to NM-GalA residues DM~27%

Re-organization of Ca^{2+} through cooperative binding

Stable crosslinks

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Blockwise de-esterified pectins: DM~27%

Low $[\text{Ca}^{2+}]$: $R = 0.24$

Cooperative binding of $\text{Ca}^{2+}$ to NM-GalA residues (stable crosslink)

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