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Stiffness of Ca²⁺-pectin gels: Combined effects of degree and pattern of methylesterification for various Ca²⁺ concentrations
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Abstract

The influence of the degree and pattern of methylesterification (DM and PM, respectively) on the stiffness of Ca^{2+} -pectin gels is extensively examined, at various Ca^{2+} concentrations. Accordingly, a highly methylesterified pectin was selectively de-esterified using NaOH, plant or fungal pectin methylesterase in order to produce series of pectins with varied pattern and broad ranges of methylesterification. The PM was quantified as absolute degree of blockiness (DB_{abs}). Ca^{2+} -pectin gels were prepared at various Ca^{2+} concentrations. Gel stiffness (G' at 1 rad/s) was determined and mapped out as a function of DM, DB_{abs} and Ca^{2+} concentration. At low Ca^{2+} concentrations, G' depends on polymer's DM and DB_{abs} . At high Ca^{2+} concentrations, a master curve is obtained over a wide range of DM, irrespective of DB_{abs} . Depending on methylesterification pattern, increase of G' is related not only to an increase in the number of junction zones per pectin chain, but also to an increase in the size of junction zones and the number of dimerised chains occurring in the gels. These results provide a detailed insight into the occurrence of junction zones in Ca^{2+} -pectin gels.

Keywords: Pectin - De-esterification - Absolute degree of blockiness - Ca^{2+} -pectin gels - "Egg-box" model - Gel stiffness

35 **List of abbreviations**

36 DB_{abs} = absolute degree of blockiness

37 DM = degree of methylesterification

38 NM- = non-methylesterified

39 GalA = D-galacturonic acid

40 NM-MDT-GalA = non-methylesterified mono-, di- and tri-GalA

41 PDP = partially de-esterified pectin

42 Endo-PG = endo-polygalacturonase (EC 3.2.1.15)

43 PME = pectin methylesterase (EC 3.1.1.11)

44

1. Introduction

Pectin is a diverse polysaccharide originating from the cell walls of mainly dicotyledonous plants. The molecular structure of this biopolymer includes, among others, a linear homopolymer of $\alpha(1-4)$ -linked D-galacturonic acid (GalA) residues, known as homogalacturonan, of which certain GalA residues can be methylesterified at the C-6 carboxyl.^{1,2} This pectic domain is usually characterised by the degree and pattern of methylesterification (DM and PM, respectively). While DM is directly calculated from the methyl group and GalA contents of pectin, the PM has been successfully quantified using indicative parameters such as “absolute degree of blockiness” (DB_{abs}).

DB_{abs} can be determined by means of enzymatic fingerprinting using endopolygalacturonases, EC 3.2.1.15 (endo-PG).^{3,4} Under suitable conditions, endo-PG extensively hydrolyses partially de-esterified pectin (PDP), yielding methylesterified galacturonides as well as non-methylesterified mono-, di- and tri-GalA (NM-MDT-GalA). Specifically, *Kluyveromyces fragilis* endo-PG, which was used in this study, hydrolyses PDPs depending on the presence of contiguous NM-GalA residues (also referred to as GalA blocks) on the polymer^{4,5}, as illustrated in Figure 1. Pasculli et al. (1991)⁵ proposed that *K. fragilis* endo-PG requires the presence of at least four consecutive GalA residues in order to cleave linkages between GalA residues (Figure 1A). However, the release of NM-galacturonides necessitates the presence of degradable blocks of at least five consecutive NM-GalA residues (Figure 1B and C). Following the release of NM-galacturonides, they are quantified and DB_{abs} is calculated as the molar ratio of the

67 total NM-GalA released as NM-MDT galacturonides to the total GalA content of the
68 polymer⁶. Hence, DB_{abs} gives an indication of the absolute occurrence of non-
69 methylesterified GalA (NM-GalA) residues in blocks (of at least five residues) over the
70 entire pectin, thus portraying the methylester distribution of the polymer.

71 Specific pectins are capable of forming gels in the presence of divalent cations, such as
72 calcium (Ca^{2+})⁷, and optimally at high pH (well above the acid dissociation constant of
73 GalA). Owing to this functional property, extracted and *in planta* pectins are used as
74 texturising polymers in numerous applications.⁸⁻¹⁰ The formation of Ca^{2+} -pectin gels is
75 by means of junction zones formation, according to the so-called “egg-box” model.^{11,12}
76 In junction zone formation, contiguous NM-GalA residues of pectin molecules are bound
77 via Ca^{2+} bridges. The strength and stability of the junction zones depend on the size of
78 the NM-GalA blocks (number of contiguous NM-GalA residues present in the blocks)
79 and the amount of Ca^{2+} available for cross-linking.^{13,14}

80 While characterising the PDPs that were used in the present study, Ngouémazong et al.
81 (2011)¹⁵ reported that, after de-esterification, both the size and the number of NM-GalA
82 blocks vary depending on the extent and mechanism of de-esterification. Specifically, at
83 DM of about 60%, large NM-GalA blocks (whose *K. fragilis* endo-PG depolymerisation
84 was similar to that of an ester-free polymer, poly-D-GalA) were already predominant in
85 PDPs de-esterified by plant pectin methylesterase EC 3.1.1.11 (PME). In NaOH and
86 fungal PME de-esterified PDPs, such large NM-GalA blocks mainly occurred from DM
87 of approximately 35%¹⁵. In addition, plant PME de-esterification has been reported to
88 result in a sequential occurrence of NM-GalA blocks on pectin chains (i.e. one chain

after the other) while fungal PME randomly produces NM-GalA blocks on pectin chains.³ These differences in pectins' PM will in turn affect the size and the number of junction zones in Ca^{2+} -pectin gels. As Ca^{2+} -pectin gels are generated through junction zones, their occurrence obviously affects gel characteristics, particularly gel stiffness (storage modulus at definite angular frequency).

So far, several studies have related structural features of pectin (DM and PM) to the storage modulus of Ca^{2+} -pectin gels.^{6,12,16-18} However, the use of a limited number of samples, a restricted DM range under study or partially de-esterified pectins produced from different starting material hampered an in-depth assessment of the combined effects of different factors on the mechanical properties of Ca^{2+} -pectin gels. For example, results from previous experimental set-ups could not be interpreted in terms of changes in the number and/or size of junction zones in Ca^{2+} gels produced from de-esterified pectins.

Therefore, this work aimed at an extensive and profound examination of the effect of pectin demethylesterification on the stiffness of Ca^{2+} -pectin gels, as a means of gaining insight in the occurrence of junction zones. Hereto, produced and characterised PDPs of broad DM range (from ~ 94% to ~ 6%) and varied patterns of methylesterification¹⁵, were used for the preparation of gels with various Ca^{2+} concentrations. The stiffness (G' at 1 rad/s) of the Ca^{2+} -pectin gels was monitored. Relating this gel property to the structural features of pectins, allowed an improved qualitative characterisation of Ca^{2+} -pectin gels, particularly, in terms of changes in the size and number of junction zones with pectin de-esterification.

2. Materials and methods

2.1 Materials

Partially de-esterified pectins, characterised in terms of molar mass distribution profile, DM and PM (the latter quantified as DB_{abs} using *K. fragilis* endo-PG) as earlier reported by Ngouémazong et al. (2011)¹⁵, were used for Ca^{2+} gel preparation. The characterised PDPs were produced from a high-DM citrus pectin (DM ~ 94%, GalA content ~ 85% on dry basis), which was purchased from Sigma (Belgium) and encoded M94. PDPs with various DM and PM were produced by controlled partial de-esterification of M94 using plant (carrot) pectin methylesterase (PME) (P-pectins), fungal (*Aspergillus aculeatus*) PME (F-pectins) or chemical saponification (NaOH) (C-pectins), as described by Ngouémazong et al. (2011).¹⁵ All chemicals used were of analytical grade.

2.2. Preparation of Ca^{2+} -pectin gels

For gel preparation, each PDP sample was dissolved in ultra pure water (organic-free, 18 MΩ.cm resistance, Millipore, USA) at 4 °C. 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 frozen and stored at -40 °C until 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 3 M $CaCl_2$ solution prepared with ultra pure water was used as $CaCl_2$ stock solution.

Prior to gel preparation, this stock solution was diluted so as to produce gels with

defined Ca^{2+} concentration, expressed as the stoichiometric ratio ($R = 2[\text{Ca}^{2+}] / [\text{COO}^-]$)¹⁹, while adding a constant volume of CaCl_2 . The actual concentration of the CaCl_2 solution hence depended on the DM of the PDP samples and the required R-value of the gels. The latter R-value was varied between 0.5 and 5.7. The final GalA content of the gels was 1.53% (w/w).

A technique involving the combination of mild heating and diffusion of Ca^{2+} through microlitres of pectin solution was used for Ca^{2+} -pectin gel preparation.²⁰ Preliminary experiments aimed at defining controlled tests conditions enabled to generate accurate results. Ca^{2+} -pectin gels were prepared directly on the lower plate of a stress-controlled Physica MCR 501 rheometer (Anton Paar, Austria). The pectin and CaCl_2 solutions were preheated to 50 °C and 30 °C, respectively. Meanwhile, the Peltier-controlled lower plate of the rheometer was preheated to 50 °C. Exactly 262 µL of preheated pectin solution was pipetted onto 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 parallel plate was then lowered to the set measuring gap. 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.

2.3 Small-amplitude oscillatory shear tests

Once loaded on the stress-controlled rheometer, the Ca^{2+} -pectin mixture was allowed to equilibrate for 10 min at 50 °C after which it was cooled from 50 °C to 20 °C within 1 h (0.5 °C/min). The gel was subsequently let to evolve for 5 h at 20 °C. Preliminary stress sweep tests (at 1 rad/s) were carried out on a number of Ca^{2+} -pectin gels in order to

define the linear viscoelastic region of the gels, in which the viscoelastic response of the sample (storage modulus, G') and loss modulus, G'') is independent of the applied stress amplitude. All subsequent oscillatory shear tests were carried out within this linear region, thereby excluding effects of shear-induced gelation or network degradation. During the cooling step and the 5 h isothermal (at 20 °C) period, a time sweep test was performed at 1 rad/s, followed by a frequency sweep test (0.1-10 rad/s) at the end of gel formation. The time evolution and frequency dependence of the moduli have already been described²⁰. From the gels' mechanical spectra, G' was determined at constant angular frequency (1 rad/s), as a measure for the stiffness of Ca^{2+} -pectin gels.^{21,22} These gel preparation method and rheological test results have been reported to be very reproducible²³.

3 Results and discussion

Characterised PDPs of various DM and PM¹⁵ were used for the preparation of Ca^{2+} -pectin gels with various Ca^{2+} concentrations, expressed as R-value. At certain lower R-values (depending on pectin type and DM), the stiffness (G' at 1 rad/s) of Ca^{2+} -pectin gels prepared from specific C-pectins (DM ~ 48, 35 and 26%) and F-pectins (DM ~ 45, 37, 29%) showed a decrease during the 5 h period set for isothermal conditions (results not shown). As proposed by Ngouemazong et al. (2012)²⁰, this decrease is related to the instability of the junction zones formed in the gels as a result of the presence (in PDP polymers) of NM-GalA blocks of a size not long enough to ensure, at low Ca^{2+} concentrations, cooperative binding of the cations. In this study, only gels which did not display decreasing gel stiffness with time (within the 5 h period) will be discussed further.

3.1 Effect of Ca^{2+} concentration on gel stiffness

The stiffness of the Ca^{2+} -pectin gels as a function of R-value as well as DM is depicted in Figure 2. The effect of DM on gel stiffness will be discussed in the next section (3.2).

The stiffness of all Ca^{2+} -pectin gels increased with R-value up to a rather constant plateau value. The initial increase of G' with increase in Ca^{2+} concentration results from additional cross-linking of NM-GalA blocks groups, as earlier explained by numerous researchers.^{18,24-28} The critical R-value at which the plateau G' was reached varied with the PM of the PDPs. In P-pectin gels, G' became independent of R-value from $R \sim 1.0$ whereas the stiffness of C- and F-pectin gels only reached a plateau at higher R-values, particularly for gels prepared from PDPs of high to intermediate DM (i.e. C66 to C26 and F64 to F36). In other words, above specific Ca^{2+} concentrations (which depend on the PM of the PDPs), additional Ca^{2+} did not cause an additional increase in the stiffness of Ca^{2+} -pectin gels. The Ca^{2+} concentrations at which the plateau G' was reached is referred to as “saturating” Ca^{2+} concentrations. Similar results (though at lower R-values, likely due to lower pectin concentrations) have been reported earlier for gels produced from NaOH-saponified pectins as well as from tomato or *A. aculeatus* PME de-esterified pectins¹⁸.

These observations indicate that, at certain Ca^{2+} concentrations (depending on the type of PDP), all possible chain associations which can contribute to the stiffness of Ca^{2+} -pectin gels may be completely formed. The variation in the critical R-values (at which the plateau G' is attained) will be explained based on the principle of junction zone formation and the structural features of the PDPs.

202 It has been reported in Ca^{2+} -pectin gelation that pectin chains are initially dimerised
203 through the binding of Ca^{2+} to GalA blocks.^{11,12,19} An eventual excess of the cation (i.e.
204 saturation of gels with Ca^{2+}) induces the aggregation of these dimerised chains via
205 weak electrostatic interactions.^{12,29} The initial formation of dimers contributes to an
206 increase of the G' of Ca^{2+} -pectin gels^{24,28}, whereas the subsequent aggregation of
207 dimers is reported to cause only a limited increase of the gels' G' .^{12,29} These literature
208 findings suggest that, above a critical R-value for Ca^{2+} saturation, all possible dimers
209 can be expected to be totally formed because NM-GalA blocks present on pectins are
210 expected to be fully cross-linked or totally filled with Ca^{2+} . In the present work, Ca^{2+}
211 saturation occurred at different R-values depending on the DM and PM of the PDPs,
212 with all P-pectin gels revealing the lowest critical R-value (i.e. ~ 1.0) as compared to the
213 gels of F- and C-pectins of intermediate to low DM.

214 At similar DM, P-pectins have been reported to be characterised by the presence of
215 larger NM-GalA blocks as compared to F- and C-pectins.¹⁵ Specifically, F- and C-
216 pectins of high to intermediate DM are characterised by high number of short to
217 intermediate NM-GalA blocks on the molecules. This suggests the presence of some
218 large methylesterified GalA blocks on F- and C-pectins. Stretches of methoxyl groups
219 have been reported to prevent the binding of Ca^{2+} to NM-GalA blocks.^{12,13,30} In fact,
220 based on the Ca^{2+} condensation theory proposed by Donati et al. (2006a,b)^{31,32}, it can
221 be suggested that, Ca^{2+} that are condensed on stretches of methoxyl groups (not
222 permanently but in a fast exchange between the polymer and the solution) are not
223 available for (complete) binding the short stretches of NM-GalA of F- and C-pectins of
224 high to intermediate DM. Moreover, in the counterion condensation theory, the free and

condensed counterions are in dynamic exchange equilibrium. This suggests that upon addition of Ca^{2+} into the system, part of the added Ca^{2+} will be condensed on the poorly accessible short NM-GalA blocks (in order to maintain the equilibrium). The affinity of the condensed Ca^{2+} toward the binding sites (carboxyl groups) will result in cross-linking and subsequent complete filling of the short NM-GalA blocks. Therefore, in F- and C-pectin gels, more cations (as compared to the amount required for P-pectin gels) will be necessary before Ca^{2+} cross-linking of short NM-GalA blocks can be (fully) achieved. At $R > 1.0$, the increase of G' in C- and F-pectin gels of high to intermediate DM indicates the formation of additional Ca^{2+} cross-links. Therefore, at a given DM and R-value, the amount of Ca^{2+} being effectively engaged in dimer formation is likely lower for F- and C-pectin gels, than for P-pectin gels.

3.2 Effect of DM on gel stiffness at various Ca^{2+} concentrations

A plot of the stiffness of P-, F- and C-pectin gels vs. DM for several R-values is given in Figure 3. Gel stiffness increased with de-esterification. However, the increasing trend varied between the three types of gels, depending on R-value. At low R-values (~ 0.5), P-pectin gels displayed the highest stiffness, whereas C-pectin gels showed the lowest, when comparing gels prepared from PDPs with similar DM (Figure 3A). As the Ca^{2+} concentration increased to $R \sim 1.0$, the stiffness of C- and F-pectin gels approached that of P-pectin gels, particularly for gels prepared from PDPs with intermediate to low DM (i.e. $< 60\%$), (Figure 3B). Interestingly, at even higher Ca^{2+} concentrations, all gels prepared from PDPs with similar intermediate to low DM showed rather comparable

stiffness (Figure 3C). Conversely, the stiffness of the gels prepared from PDPs with high DM (> 60%) was distinctly different, with C-pectin gels showing the lowest G' .

At low R-values, the lower stiffness of F- and C- pectin gels as compared to that of P- pectin gels of similar DM is an indication of the strong gelling behaviour of P-pectins, as reported by a number of researchers.¹⁶⁻¹⁸ In fact, as earlier suggested by some researchers, pectins with larger size of NM-GalA blocks such as P-pectins, bind Ca^{2+} more strongly than those with shorter NM-GalA blocks such as F- and C-pectins.^{17,33,34} At saturating Ca^{2+} concentrations, the similar gel stiffness displayed by all gels of similar DM (except mainly C-pectins with DM > 60%), indicates the limited effect of PM on gel stiffness. Therefore, we suggest that, when Ca^{2+} availability is no longer a limiting factor for gelation, DM rather than PM plays the major role in controlling the stiffness of Ca^{2+} -pectin gels prepared from PDPs with intermediate to low DM. The lowest G' displayed by C-pectin gels of DM > 60% is likely related to the absence or extremely limited occurrence of NM-GalA blocks on these PDPs (extremely low DB_{abs} values (i.e. ~0.26) as opposed to the sparsely occurring NM-GalA blocks on P-and F-pectins (DB_{abs} ~5%)).

3.3 Effect of DB_{abs} on gel stiffness at various Ca^{2+} concentrations

The effect of DB_{abs} on Ca^{2+} -pectin gel stiffness is illustrated in Figure 4. Gel stiffness gradually increased with polymer DB_{abs} , irrespective of R-value, for the three types of gels. Nonetheless, some differences were observed depending on R-value.

At low R-value ($R \sim 0.5$), the stiffness of the gels prepared from PDPs of different type but with similar DB_{abs} was rather comparable (Figure 4A). As the Ca^{2+} concentration increased ($R \sim 1.0$), only small differences in gel stiffness were observed (Figure 4B).

268 Interestingly, at even higher R-value (R-values at Ca^{2+} saturation), pronounced
269 differences were noticed, particularly between the stiffness of P-pectin gels and those of
270 C- and F-pectin gels (Figure 4C). Actually, the stiffness of C- and F-pectin gels showed
271 a rather parallel increasing trend with DB_{abs} , contrary to P-pectin's gel stiffness. At DB_{abs}
272 $< 20\%$, the stiffness of C- and F-pectin gels displayed a steep increase with DB_{abs}
273 whereas, at $\text{DB}_{\text{abs}} \geq 20\%$, a limited increasing trend was observed. In P-pectin gels, G'
274 displayed a more moderately increasing trend for DB_{abs} up to higher values (DB_{abs}
275 $\sim 50\%$), where it became similar to that of C- and F-pectin gels.

276 At low and similar DB_{abs} ($\text{DB}_{\text{abs}} < 20\%$), the remarkable higher G' value of the gels
277 prepared from C- and F-pectins compared to those of P-pectin gels is likely due to the
278 presence of more junction zones (albeit shorter) in C- and F-pectin gels. This is
279 consistent with the findings that a high molar concentration (or low molar mass) of
280 elastically active network chains (i.e. the portion of pectin chain that is located between
281 two junction zones) results in high G' values.^{25,35}

282 Prior to further discussion of these results, it is worth noting that PDPs with similar DB_{abs}
283 carry NM-GalA blocks of which the combination of size and number results in the
284 release of similar amounts of total NM-galacturonides, although the actual size and
285 number of the blocks can differ (see calculation of DB_{abs} in section 1). The first step in
286 Ca^{2+} -pectin junction zone formation is the dimerisation (resulting in increase in gel G') of
287 the NM-GalA blocks of the polymer, which is supposed to be complete at saturating
288 Ca^{2+} concentrations. When all existing NM-GalA blocks would be dimerised, the
289 differences in the size and number of NM-GalA blocks per chain as well as the number
290 of chains bearing NM-GalA blocks would result in similar differences in the size and

number of junction zones per pectin chain as well as the number of Ca^{2+} -dimerised chains in the gel. A closer look at the effect of the methylester (thus NM-GalA block) distribution pattern (quantified as DB_{abs}) on Ca^{2+} -pectin gel stiffness (G') shown in Figure 4C, can then enable to provide a qualitative evaluation of the changes in the size and number of junction zones in Ca^{2+} -pectin gels, prepared from the three types of PDPs (P-, F- and C-pectins).

3.3.1 Changes in the size and number of junction zones in Ca^{2+} -pectin gels prepared from randomly de-esterified pectins (C- and F-pectins)

The structural characteristics of the PDPs used for gel preparation have been reported earlier¹⁵. C-pectins of (very) high DM ($\sim 94\% > \text{DM} > 50\%$ i.e. $\text{DB}_{\text{abs}} < 9\%$) exhibited a large number of short NM-GalA blocks with less than five NM-GalA residues. C-pectins of intermediate to low DM ($50\% > \text{DM} > 35\%$, i.e. $9\% < \text{DB}_{\text{abs}} < 20\%$) bore, besides the short NM-GalA blocks, sparsely occurring NM-GalA blocks with at least six NM-GalA residues. C-pectins of low DM ($\text{DM} \leq 35\%$, i.e. $\text{DB}_{\text{abs}} \geq 20\%$) were characterised by the presence of NM-GalA blocks with at least seven NM-GalA residues. The structural features of these PDPs imply that, at saturating Ca^{2+} concentrations, C-pectins with $\text{DB}_{\text{abs}} < 20\%$ generate gels predominantly characterised by a high number of short junction zones, whereas PDPs with $\text{DB}_{\text{abs}} \gg 20\%$ yield gels consisting of larger junction zones. This implies that, in the gels prepared from C-PDPs with $\text{DB}_{\text{abs}} < 20\%$, the pronounced increase in gel stiffness with DB_{abs} (Figure. 4C) can be assumed to result mainly from an increase in the number of junction zones per polymer chain whereas, for

gels of C-PDPs with $DB_{abs} \geq 20\%$, the limited further increase of the gel stiffness with polymer DB_{abs} results from an increase in the size of the existing junction zones.

The observed parallel increasing trend of the stiffness of C- and F-pectin gels with DB_{abs} , indicates that the changes in the size and/or number of junction zones is likely similar in both gel types. However, at similar DB_{abs} (for $DB_{abs} < 20\%$), C-pectins revealed a higher proportions of NM-mono-GalA as compare to F-pectins. Hence, the former PDPs displayed shorter GalA blocks than the latter.¹⁵ This suggests that, at saturating Ca^{2+} concentrations, C-pectin gels contain a higher number of shorter junction zones while F-pectin gels reveal a smaller number of larger junction zones. Therefore, at $DB_{abs} < 20\%$, the somewhat higher G' of C-pectin gels as compared to that of F-pectin gels (Figure 4C) implies that a high number of short junction zones per polymer chain has a greater contribution to gel stiffness than a small number of large junction zones. In the same context, Vincent and Williams (2009)³⁶ reported while carrying out microrheological studies on calcium-induced pectin gels that, the elastic properties of these gels can be maximized by increasing the number of junction zones (those that are just long enough to give stable cross-links).

A schematic representation of the proposed changes in the number and size of junction zones with DB_{abs} is given in Figure 5, for Ca^{2+} -pectin gels prepared from randomly de-esterified pectins at saturating Ca^{2+} concentrations. In summary, gels prepared from pectins obtained at successive stages of random de-esterification initially display an increasing number of short junction zones followed by an increasing size of the existing junction zones.

3.3.2 Changes in the size and number of junction zones in Ca²⁺-pectin gels prepared from blockwise de-esterified pectins (P-pectins)

As reported earlier by Ngouémazong et al. (2011)¹⁵, P-pectins with very high DM (DM ≥ 80%, i.e. DB_{abs} ≤ 5%) were characterised by few relatively large NM-GalA blocks with at least six NM-GalA residues. P-pectins with high DM (80% > DM > 57%, i.e. 5 < DB_{abs} < 33%) exhibited GalA blocks of increased size and/or number per polymer chain. This suggests that, at saturating Ca²⁺ concentrations, gels prepared from a PDP with DB_{abs} of ~5% revealed few relatively large junction zones, whereas the gels prepared from the PDPs of DB_{abs} between 5 and 33% have larger and sometimes more junction zones per polymer chain.

P-pectins of low DM (DM ≤ 57%, i.e. DB_{abs} ≥ 33%) bore large NM-GalA blocks, followed by an enlargement of the existing NM-GalA blocks and/or a further augmentation in the number of large NM-GalA blocks. As seen previously for C- and F-pectin gels (section 3.3.1), an increase in the size of junction zones only results in a limited increase in gel stiffness. Still, in Ca²⁺-pectin gels prepared from P-pectins, gel stiffness showed a remarkable increase with DB_{abs} (up to DB_{abs} ~50%) (Figure 4C). This suggests that these gels are prepared from PDPs which display not only an increase in the size of NM-GalA blocks per pectin chain, but also an increase in the number of large NM-GalA blocks in the polymer. However, as the increasing trend of G' with DB_{abs} for P-pectin gels is different from the one of C- and F-pectin gels, it is suggested that the former is associated with an augmentation of the number of pectin chains bearing large NM-GalA blocks. This is consistent with the reports of Luzio and Cameron (2008)³³ in which the high G' of a Ca²⁺-pectin gel prepared from a citrus PME de-esterified pectin (50% DM)

was attributed to the incorporation of a large proportion of molecules in the gel structure. Hence, P-pectin gels prepared from PDPs with $DB_{abs} \geq 33\%$ presumably have large junction zones on an increasing number of polymer chains. Therefore, the increase in the stiffness of P-pectin gels with DB_{abs} (Figure 4C) is mainly associated with an increase in the size of junction zones per pectin chain as well as with an increase in the number of Ca^{2+} -dimerised chains in the gel. Nonetheless, at $DB_{abs} \geq 50\%$ (DM $\sim 37\%$), further increase in DB_{abs} only had a limited additional effect on the stiffness of P-pectin gels. This observation suggests that when a high number of pectin chains are dimerised, the formation of additional dimers only has a limited effect on gel stiffness.

A schematic representation of the proposed changes in the number and size of junction zones in Ca^{2+} -P-pectin gels with increasing DB_{abs} at saturating Ca^{2+} concentration is given in Figure 6. In summary, gels prepared from P-pectins obtained at successive stages of de-esterification display mainly an increasing size of the junction zones per polymer chain followed by an increasing number of dimerised chains in the gels.

Furthermore, the comparable moduli observed in gels prepared from C-, F- and P-pectins with $DB_{abs} \geq 50\%$ may indicate that either the corresponding gels have comparable sizes and numbers of junction zones (irrespective of the number of chains that have been really dimerised), or the different combinations of sizes and numbers of junction zones per pectin chain as well as number of dimerised chains result in similar net effects on gel stiffness.

4 Conclusion

Selected characterised PDPs were used for the preparation of Ca^{2+} -pectin gels with various Ca^{2+} concentrations and the stiffness of the resulting gels was measured. At low Ca^{2+} concentration, gels prepared from C-pectins displayed the lowest stiffness at a given DM, whereas P-pectin gels showed the highest. Gel stiffness was then mainly determined by DM and DB_{abs} . At high Ca^{2+} concentration and at a given DM, gel stiffness was independent of PM, for a wide DM range (intermediate to low). Furthermore, the evolution of gel stiffness with DB_{abs} at saturating Ca^{2+} concentrations was linked to changes in the size and number of junction zones in the gel. We proposed that, in C- and F-pectin gels, increase in stiffness was mainly linked to the increase in the number of short junction zones per polymer chain whereas, in P-pectin gels, increase in stiffness was generally related to an increase in the size and number of junction zones per polymer chain as well as the number of dimerised chains in the gels. Therefore, beside Ca^{2+} concentration, both DM and DB_{abs} must be considered in order to fully understand the rheological behaviour of Ca^{2+} -pectin gels.

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Figures

Simplified illustration of the principle of the release of non-methylesterified galacturonides from PDPs by the endo-PG of *K. fragilis*. The thick downward arrow shows the proposed depolymerisation site of endo-PG. (A) Required number of contiguous GalA for endo-PG depolymerisation. (B) and (C) required number of contiguous GalA for release of non-methylesterified galacturonides, for GalA blocks with ≤ 7 and > 7 GalA residues, respectively (adapted from Daas et al. (2000))⁴.

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476 **Ngouémazong et al. Figure 1**

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Gel stiffness (G' at 1 rad/s) of Ca^{2+} -pectin gels as a function of Ca^{2+} concentration (expressed as R-value). The dotted line indicates the R-value at which P-pectin gels reach the plateau level of gel stiffness.

Ngouémazong et al. Figure 2

Effect of DM on the stiffness (G' at 1 rad/s) of P- ($\square$), F- ($\circ$) and C- (Δ)pectin gels. A, B and C represent $R \sim 0.5$, ~ 1.0 and at Ca^{2+} saturation of gels, respectively. Experimental points are linked with a line.

Ngouémazong et al. Figure 3

Effect of DB_{abs} on the stiffness of P- ($\square$), F- (o) and C-(Δ) pectin gels at $R \sim 0.5$ (A), $R \sim 1.0$ (B) and R at Ca^{2+} saturation (C).

Ngouémazong et al. Figure 4

Schematic representation of the proposed occurrence of junction zones in Ca^{2+} -pectin gels prepared from randomly de-esterified pectins at saturating Ca^{2+} concentration (changes in the size and number of junction zones is shown from top to bottom). It should be noted that the figure illustrate de-esterification and junction zone formation. It does not represent the real position of pectin chains with respect to each other.

Ngouémazong et al. Figure 5

Schematic representation of the proposed occurrence of junction zones in Ca^{2+} -pectin gels prepared from plant PME de-esterified pectins at saturating Ca^{2+} concentration. It should be noted that the figure illustrate de-esterification and junction zone formation. It does not represent the real position of pectin chains with respect to each other.

Ngouémazong et al. Figure 6